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SELECTION LIMITS WITH SIBMATINGS IN DROSOPHILA MELANOGASTER

A. O. TANTAWY[1]

Faculty of Agriculture, University of Alexandria, Egypt, U.A.R.

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EXPERIMENTS with Drosophila have been undertaken by many investigators to study the effects of selection on different quantitative characters. In such experiments, the parents of the next generation are always selected because they show a deviation in the direction desired from their population mean. A survey of the earlier investigations has been given by TANTAWY (1956a, b) using wing length in *Drosophila melanogaster* as the selection criterion. Other analyses of selection phenomena have been published by CLAYTON *et al.* (1957a b c), FALCONER and A. ROBERTSON (1956), PREVOSTI (1956), RASMUSON (1956), SCOSSIROLI (1956), and MARIEN (1958). They all agree that selection is effective in plus and minus directions; the latter showing greatest deviations from the initial population. The results demonstrate that the response to selection is limited; sooner or later selection limits are reached beyond which the character cannot be altered. It has been also shown that back selection and relaxed selection in negative and positive directions may result in rapid return to the control level in some experiments (TANTAWY 1956) and ROBERTSON and REEVE (1952) but not in others (TANTAWY 1956a). Recently, LERNER (1958) discusses the quantitative aspects of selection and presented the subject in a very lucid and full manner.

The present experiments were designed to provide information as to how long the response to selection may continue in highly inbred lines of *Drosophila melanogaster*.

MATERIALS AND TECHNIQUES

The stock used for the present experiments was the Carianlarich stock which has been kept by mass mating of 20 pairs of flies per bottle in every generation.

Technical procedures followed in the present experiments for control of environmental variations, matings between selected parents, method of selection and estimation of the heritability of body size, were similar to those reported previously by the author (1956a, 1957a). Experiment I with lines A and C, and experiment II with lines B and D were carried out during the years 1955 and 1956, respectively. In both experiments, lines A,B and C,D were maintained by selection for long and short wing length. In all the lines, matings between brothers and sisters were made. A control stock had been kept by mass mating for each experiment under environmental conditions similar to those of the selected lines.

[1] Present address: Department of Zoology, Columbia University, New York, N.Y., U.S.A.

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Selection in both experiments was relaxed at the first, seventh, tenth and twentieth generations, at which times coefficients of inbreeding should have been about 25, 78, 88 and 99 percent, respectively, for five successive generations. Heritability of the body size was estimated first for the initial foundation population, and then at the different levels of homozygosity indicated above.

RESULTS

Heritability of wing and thorax length in the initial foundation population

Heritability of body size *i.e.*, wing and thorax length, in the initial foundation population was estimated by the method of the regression of offspring on mid-parent as reported by TANTAWY (1957a). Six progeny tests were carried out, three of which involved random matings and three involved assortative matings, the latter being corrected for the magnified variance between the parents as indicated by REEVE (1953).

TABLE 1

Heritability of wing and thorax length, with their respective standard errors, and the genetic correlation between them in the initial foundation population

Progeny test no.	Mating system	Heritability percent		No. of matings	Genetic correlation percent
		Wing length	Thorax length		
1st (Oct. 1954)	Random	41.25±4.50	46.50±5.20	100	84
2nd (Feb. 1955)	Random	47.32±4.05	48.21±3.12	100	78
3rd (Oct. 1955)	Random	43.52±3.21	45.05±3.52	100	81
4th (July 1955)	Assortative	45.00±6.06	47.12±6.05	100	83
5th (Feb. 1956)	Assortative	41.12±5.30	42.06±4.51	97	80
6th (July 1956)	Assortative	41.01±6.57	43.00±5.01	98	79

The results obtained from the various progeny tests and the respective standard errors are presented in Table 1, where the data for each progeny test are given. The weighted means for all tests show that 43 and 45 percent of the total variance for wing and thorax length, respectively, are apparently due to the additive genetic variance. After correction for the magnified variance between the parents in the assortative test, the average genetic correlation was found to be 80.3 percent.

These results agree fairly well with those reported by REEVE and ROBERTSON (1953), TANTAWY (1957a, b) and TANTAWY and REEVE (1956) who showed that the additive genetic variance for wing length in *Drosophila melanogaster* ranged between 30 percent and 45 percent.

Selection responses in wing and thorax length

Selection responses in all selected lines were measured as their deviations from the control stock of a mass mated population reared under similar environmental conditions as the experimental lines.

TANTAWY (1956b) has shown the absolute size of the Carianlarich population of *Drosophila melanogaster* which has been used in the present study. The results obtained with both sexes were averaged since no differences between sexes have been observed. The main features of the response to selection for wing length are presented in Figure 1.

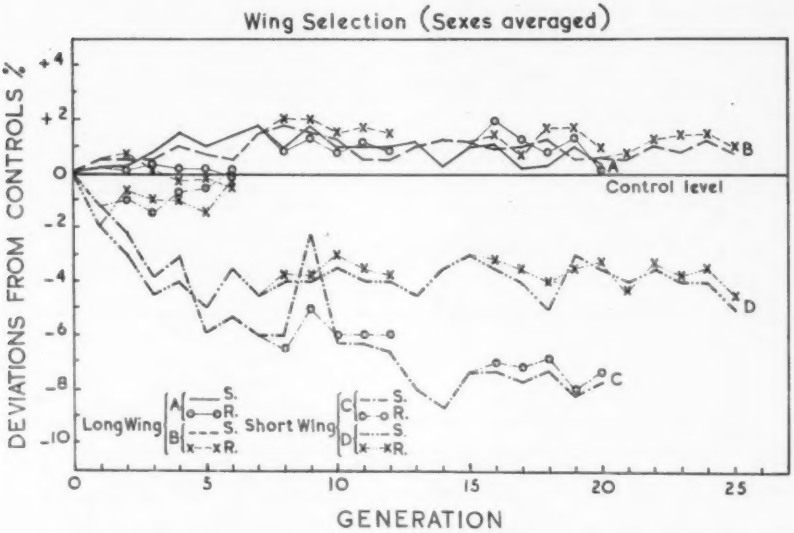

FIGURE 1.—Percentage deviations of wing length from controls, sexes averaged, where A,B,C,D, are the selected lines and S=selected and R=relaxed.

Examination of Figure 1 shows that selection is effective in both plus and minus directions, but that selection for small size shows greater response than that for large size. Figure 1 also demonstrates that lines A and B, selected for long wing, followed almost the same general trend. In both lines selection was effective to the fourth generation; they showed 1.5 and 1.0 percent deviations from the control level, respectively; the lines then remained almost constant throughout the experiments.

Lines C and D selected for short wings behaved in a different manner. Both lines responded similarly to selection for the first five generations and then diverged with line C (shown in Figure 1) producing a greater response. Selection was effective in both lines to the fifth generation, after which there was a tendency for them to remain stable to the end of the experiments. Such differences in the response to selection after the fifth generation between lines C and D could be attributed to some environmental factors which affect the selected and the control lines differently.

Similar results were achieved also for thorax length (results not included); this is to be expected because of the high positive genetic correlation between wing and thorax measurements (Table 1).

Phenotypic variance

Phenotypic variation for wing length is expressed as coefficients of variation, and the results are presented in Figure 2 for experiments I and II.

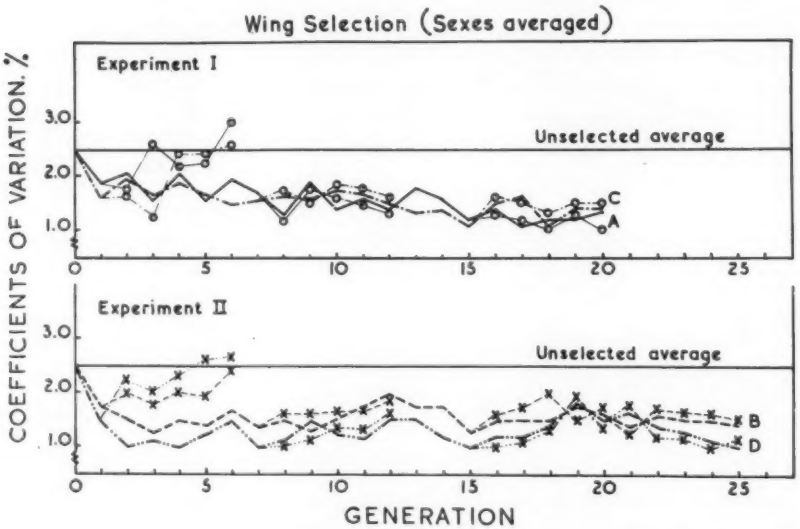

FIGURE 2.—Coefficients of variation for wing length (sexes averaged) in the different selected lines. For symbols see Figure 1.

It is clear that intensive inbreeding in both experiments caused a reduction in the phenotypic variation of the selected inbred lines. Lines selected for short wing length are less variable than those selected for long wing. In all the lines, phenotypic variation declines in the earlier generations of inbreeding and then stabilizes. Thorax length behaves similarly as wing length (unpublished material).

Relaxed selection

The effects of relaxed selection on wing length, as shown in Figure 1 at different coefficients of inbreeding, indicate that relaxed selection at 25 percent coefficient of inbreeding causes an increase in the phenotypic variance to the control level in all the selected lines. At higher levels of inbreeding, relaxed selection has no effect whatsoever on the response, *i.e.* relaxed selected lines remain almost constant.

Heritability estimates in the selected lines

The heritability of wing length can be estimated as the ratio of rate of advance per generation to selection differential, the latter being the average deviation of parents from the mean size of their generation (ROBERTSON and REEVE 1952; TANTAWY 1956a). The results are presented in Table 2.

TABLE 2

Heritability of wing length in the selected lines

a. Lines A and C.

Generation	Selection differential		Advanced per generation		Heritability percent	
	Long A	Short C	Long A	Short C	Long A	Short C
0–5	3.18	—2.83	0.85	—1.71	26.7	60.4
6–10	3.18	—3.23	—0.10	—1.08	—3.1	33.4
11–15	3.73	—3.99	—0.20	—1.61	—5.4	40.4
16–20	2.75	—1.99	—0.30	—0.04	—1.1	2.0

b. Lines B and D.

	Long B	Short D	Long B	Short D	Long B	Short D
0–5	3.15	—2.95	0.78	—1.65	24.7	55.9
6–10	2.80	—3.06	0.02	—1.00	0.7	32.6
11–15	3.05	—2.85	—0.25	—1.53	—8.2	53.7
16–20	2.50	—3.05	—0.05	—0.25	—2.00	8.2
21–25	2.21	—2.75	0.11	—0.21	4.9	7.6

Table 2 shows that the heritability of wing length in the long wing selected lines declined after the first five generations to insignificant values, while in short wing selected lines heritability estimates for wing length showed higher values up to the fifteenth generation.

The regression method was used to estimate more accurately the heritability of wing length in the various selected lines, at different levels of homozygosity. The results secured from such analyses are presented in Table 3. It will be noticed

TABLE 3

Heritability of wing length, by the regression method, in the selected lines at different levels of inbreeding

Generation	Coefficients of inbreeding percent	Experiment I				Experiment II			
		Long		Short		Long		Short	
		h^2	D.F.	h^2	D.F.	h^2	D.F.	h^2	D.F.
1	25.00	38.5±5	18	42.3±4	18	35.2±4	18	40.5±3	18
5	67.2	19.4±6	18	23.2±7	18	17.2±4	18	25.3±5	18
10	88.6	6.2±4	18	10.3±6	18	5.3±2	18	10.2±3	18
15	96.1	4.3±4	16	7.6±4	18	4.2±3	14	8.3±2	16
20	98.6	3.2±2	16	5.6±3	16	6.2±4	16	5.3±4	16
25	99.5	....	..	....	..	3.1±2	14	4.3±3	16

in Table 3 that heritability of wing length declined in all lines after the fifth generation of inbreeding, i.e., after 67.2 percent coefficient of inbreeding.

The most interesting feature of the results as presented in Table 2 and 3 is the contrast in the estimates of heritability, particularly in the short wing selected lines, according to whether heritability is computed from the ratio of advance per generation to selection differential or from the regression of offspring on mid-parent size. The higher values in Table 2 reflect the decline in size due to inbreeding which is confounded with the effects of selection. The long wing selected lines show better agreement between the alternative estimates.

Effects of relaxed selection on the heritability estimates of wing length are shown in Table 4. The data in Table 4 indicate that the heritability declines

TABLE 4

Heritability of wing in the selected lines before and after relaxation at different levels of inbreeding

Coefficients of inbreeding	Before relaxation				After relaxation			
	Experiment I		Experiment II		Experiment I		Experiment II	
	Long	Short	Long	Short	Long	Short	Long	Short
25.0*	38.5±5	42.3±4	35.2±4	40.5±3	40.2±5	44.2±3	45.2±3	50.2±4
78.5*	12.8±3	18.3±6	10.2±3	32.2±5	10.3±4	15.3±4	12.3±4	10.3±5
88.6*	6.2±4	10.3±6	5.3±3	10.2±3	6.2±3	8.3±4	4.4±4	8.2±2
98.6†	3.2±2	5.6±3	6.2±4	5.2±4	4.0±2	3.0±2	5.2±3	5.5±3

* D.F. = 18.
† D.F. = 16.

greatly at higher levels of homozygosity, but at lower levels, i.e., 25 percent coefficients of inbreeding, there is practically no change in genetic variance from the initial foundation population.

Relaxed selection at the lower level of inbreeding causes an increase in the heritability estimates of the selected lines. At higher levels of inbreeding, relaxed selection has no effects on the genetic variance in the selected lines, i.e., heritability estimates remain almost constant.

Percentage emergence

Percentage emergence is calculated as the number of adults expressed in percentage of the number of eggs deposited in each vial for each line on four successive days. The results are presented in Figure 3.

Figure 3 shows that percentage emergence in the various selected lines was affected greatly at the lower coefficients of inbreeding after which it remains almost constant throughout the experiments. In both experiments, short wing selected lines showed less percentage emergence than long wing selected ones, which agrees with the results of most selection and inbreeding experiments on Drosophila.

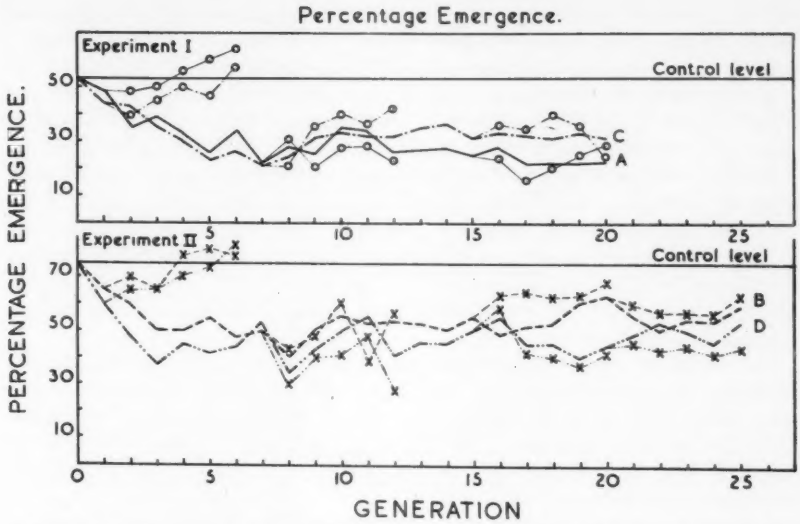

FIGURE 3.—Percentage of eggs yielding flies in the different selected lines. For symbols see Figure 1.

Relaxed selection, at lower levels of inbreeding, caused an increase in percentage emergence in all lines almost to the control level, while at higher levels of inbreeding there are practically no differences in percentage emergence between selected and relaxed lines.

SUMMARY AND CONCLUSIONS

1. Selection experiments in *Drosophila melanogaster* have been carried out with brother-sister matings for 20 and 25 generations in two identical experiments. Selection was practiced for long and short wing length and response to selection was achieved from the first generation of selection to the fourth in the long wing selected lines and to the fifth in the short wing selected lines, after which selection limits were eventually reached. Lines selected for short wing length showed higher response to selection than those selected for long wing.

2. Relaxed selected lines returned to the control level when selection was relaxed at 25 percent coefficient of inbreeding. At higher levels of inbreeding relaxed selection had no effects on the selected characters, i.e., relaxed selected lines remained almost as constant as the selected lines.

3. In all lines, thorax length behaved in the same manner as wing length with respect to all characters studied.

4. Phenotypic variation was decreased by inbreeding and selection from the first generation of selection, then ceased to decrease when body size had reached

selection limits. When selection was relaxed, phenotypic variance returned completely, at the lower levels of inbreeding, to the control level, while at higher levels, it remained almost constant in the relaxed selected lines.

5. Selection with sibmatings cannot change the genetic variance of the selected inbred lines at the lower coefficients of inbreeding. At higher levels, heritability estimates declined almost to insignificant values. When selection was relaxed, the heritability estimates increased in the relaxed selected lines at the lower coefficients of inbreeding; but at the higher levels such estimates remained constant.

6. Percentage emergence was decreased from the first generation of inbreeding and selection, and remained constant when selection limits were achieved in wing length. Such a character returned back to the control level when selection was relaxed at lower levels of inbreeding, while at higher levels, relaxed selection has no effect on such character.

The results secured in the present experiments confirm those reported by the author previously (1956a, 1957a). Selection combined with an intensive inbreeding is effective in the earlier generations. This is followed by a stabilization, for the character under selection. The stabilization is achieved owing to fixation of alleles favored by the selection which were present in the original population. Therefore, it can be concluded that:

a. Much improvement for a given trait is possible under selection with sibmatings before homozygosity for specific combining genes is approached.

b. Limits of improvements are not likely to be reached until the segregating genes become homozygous for those alleles which combine best in the selected lines.

c. Inbreeding is the major factor in eliminating heterozygosity, while selection serves to identify the genotypes favoring the progress in the desired direction.

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THE COMPONENTS OF VARIATION AMONG FAMILY MEANS IN DIALLEL CROSSES

J. L. JINKS AND J. M. STEVENS[1]

A.R.C. Unit of Biometrical Genetics, Department of Genetics, University of Birmingham

Received February 6, 1958

IN the practical application of diallel analyses, deviations from nonindependence of the genes in action and distribution have until now been inferred from the heterogeneity of the additive and dominance components estimated on the assumption of independence. HAYMAN (1957) using a method based on the C scaling test of MATHER (1945) has, however, obtained a measure of the effects due to nonallelic interactions and apparent dominance. More recently, JINKS and MORLEY JONES (1958) have considered the estimation and interpretation of the additive, dominance and nonallelic interaction components of the generation means for any degree of correlated gene distribution in the parental lines of a cross between two inbred lines. The method was illustrated by an analysis of the generation means of the parents, F_1, F_2 and backcross generations of a diallel set of crosses between eight inbred lines of *Nicotiana rustica* (JINKS 1954, 1956). In these illustrations the crosses were treated as independent. We will now consider the information to be gained by recognizing their interrelationships as members of a diallel set of crosses and show how we may estimate the additive, dominance and nonallelic interaction components of variation and recognize some of the consequences of correlated gene distributions.

Notation

In considering a diallel set of crosses we must amend the expectations used by JINKS and MORLEY JONES for independent crosses to those given by ANDERSON and KEMPTHORNE (1954). This allows for interactions between genes by which two parents differ and genes which are identical in the two parents but which are represented by different alleles in other parents. The changes involved can be illustrated by reference to the additive component of the means.

Following the notation of HAYMAN and MATHER (1955) for a pair of interacting genes *A-a*, *B-b* we previously wrote.

$$\overline{B}_1 - \overline{B}_2 = \pm d_a \pm d_b \qquad \text{for the association cross } AABB \times aabb$$
$$\text{and} \quad = \pm d_a \mp d_b \qquad \text{for the dispersion cross } AAbb \times aaBB,$$

while for the rest, where only *A* or *B* was segregating

$$\overline{B}_1 - \overline{B}_2 = \pm d_a \text{ or } \pm d_b$$

[1] Permanent address: Dept. of Genetics, Stellenbosch University, S. Africa.

We must now write the latter expectation as

cross ⧹	statistic $\overline{B}_1 - \overline{B}_2 = [d]$
$AABB \times AAbb$	$\pm\ (d_b + i_{ab} - \frac{1}{2}j_{b/a})$
$AABB \times aaBB$	$\pm\ (d_a + i_{ab} - \frac{1}{2}j_{a/b})$
$aabb \times AAbb$	$\pm\ (d_a - i_{ab} - \frac{1}{2}j_{a/b})$
$aabb \times aaBB$	$\pm\ (d_b - i_{ab} - \frac{1}{2}j_{b/a})$

Similarly our estimate of the dominance component of the generation means become:

cross ⧹	statistic $\overline{F}_1 - 4\overline{F}_2 - \frac{1}{2}\overline{P}_1 - \frac{1}{2}\overline{P}_2 + 2\overline{B}_1 + 2\overline{B}_2 = [h]$
$AABB \times aabb$ or $AAbb \times aaBB$	$h_a + h_b$
$AABB \times AAbb$	$h_b - \frac{1}{2}l_{ab} + j_{a/b}$
$AABB \times aaBB$	$h_a - \frac{1}{2}l_{ab} + j_{b/a}$
$aabb \times AAbb$	$h_a - \frac{1}{2}l_{ab} - j_{b/a}$
$aabb \times aaBB$	$h_b - \frac{1}{2}l_{ab} - j_{a/b}$

While for the nonallelic interaction components the expectations remain unaltered as:

Statistic ⧹ cross	$AABB \times aabb$	$AAbb \times aaBB$	All other crosses
$2\overline{B}_1 + 2\overline{B}_2 - 4\overline{F}_2$	i_{ab}	$-i_{ab}$	0
$\overline{P}_1 + \overline{P}_2 + 2\overline{F}_1 + 4\overline{F}_2 - 4\overline{B}_1 - 4\overline{B}_2$	l_{ab}	l_{ab}	0
$2\overline{B}_1 - \overline{P}_1 - 2\overline{B}_2 + \overline{P}_2$	$\pm j_{a/b} \pm j_{b/a}$	$\pm j_{a/b} \mp j_{b/a}$	0

We can now arrive at the expectation for an analysis of the diallel tables, which are derived by substituting for the generation means of previous analysis the estimated components of these means.

The additive component

For each cross of a diallel set the value of $\overline{B}_1 - \overline{B}_2$ may be entered in the appropriate place in the diallel table. In the absence of nonallelic interactions the expectations for the analysis of this derived diallel table are given in Table 1 (JINKS 1956). Only the heritable portion of the variation is considered. α, β, γ and δ are the frequencies of *AABB*, *AAbb*, *aaBB* and *aabb* parental genotypes respectively. Hence $4(\alpha\delta\text{-}\beta\gamma) = C$ is the correlation between the distribution of the genes in

TABLE 1

Analysis of the additive component in the absence of nonallelic interactions

Statistic	Expectations: Correlated gene distribution	Expectations: Random gene distribution	Symbols
Mean	0	0	$[\bar{d}]$
Mean variance of arrays	$\sum u_a v_a d_a^2 + u_b v_b d_b^2 + 2(\alpha\delta\text{-}\beta\gamma) d_a d_b$	$\sum u_a v_a d_a^2$	$\frac{1}{4}D$
Variance of array means	$\sum u_a v_a d_a^2 + u_b v_b d_b^2 + 2(\alpha\delta\text{-}\beta\gamma) d_a d_b$	$\sum u_a v_a d_a^2$	$\frac{1}{4}D$
Total variance	$2\sum u_a v_a d_a^2 + u_b v_b d_b^2 + 2(\alpha\delta\text{-}\beta\gamma) d_a d_b$	$2\sum u_a v_a d_a^2$	$\frac{1}{2}D$

the parental lines having a maximum value of 1 or -1 for complete correlation and 0 for no correlation i.e. random gene distribution.

The expectation for the heritable component of the array variances are constant over arrays and the variance of array means irrespective of random or non-random gene distributions. Hence all are homogeneous in the absence of non-allelic interactions. They cease to be homogeneous, however, in the presence of *j* type nonallelic interactions. Thus in the presence of nonallelic interactions the mean value of the heterogeneous array variances becomes $\Sigma u_a v_a d_a^2 + u_b v_b d_b^2 + 2(\alpha\delta\text{-}\beta\gamma)\, d_a d_b + \tfrac{1}{4}[\alpha\gamma(1+v_b) + \beta\delta(1+u_b)]\, j_{a/b}^2 + \tfrac{1}{4}[\alpha\beta(1+v_a) + \gamma\delta(1+u_a)]\, j_{b/a}^2 - \tfrac{1}{2}[\alpha\gamma(\beta\text{-}\delta) + \beta\delta(\alpha\text{-}\gamma)] j_{a/b} \cdot j_{b/a} + (\beta+\gamma)(\alpha+\delta)\ i_{ab}^2 + 2(\alpha\gamma\text{-}\beta\delta)\ d_a i_{ab} + 2(\alpha\beta\text{-}\gamma\delta)\ d_b i_{ab} - (\alpha\gamma+\beta\delta)\ d_a \cdot j_{a/b} - (\alpha\beta+\gamma\delta)\ d_b \cdot j_{b/a} - (\alpha\gamma\text{-}\beta\delta)\ j_{a/b} \cdot i_{ab} - (\alpha\beta\text{-}\gamma\delta)\ j_{b/a} \cdot i_{ab}$.

This is now greater than the expected variance of array means by $\Sigma\tfrac{1}{2}[\alpha\gamma(\beta+\delta) + \beta\delta(\alpha+\gamma)]\, j_{a/b}^2 + \tfrac{1}{2}[\alpha\beta(\gamma+\delta) + \gamma\delta(\alpha+\beta)]\, j_{b/a}^2 - [\alpha\gamma(\beta\text{-}\delta) - \beta\delta(\alpha\text{-}\gamma)]\, j_{a/b} \cdot j_{b/a}$.

With uncorrelated gene distributions this difference becomes $\Sigma\tfrac{1}{2} u_a u_b v_a v_b\ (j_{a/b}^2 + j_{b/a}^2) - u_a u_b v_a v_b\ (u_a - v_a)(u_b - v_b)\ j_{a/b} \cdot j_{b/a}$. having a maximum value of $1/32\, J$ (see later) with equal gene frequencies, i.e. $u_a = u_b = v_a = v_b$ etc.

Clearly, in the presence of nonallelic interactions we can no longer estimate the contribution of an additive component of variation depending solely on Σd^2. Furthermore, this situation is unlikely to be materially changed even if information from more generations than those considered here were made available. We might, therefore, at a later stage, profitably consider the utility of a component, which while depending on the joint effects of additive and *i* and *j* type interactions contributions, may nevertheless have some predictive value.

The dominance component

An analysis of a derived diallel table of the dominance component of the generation means gives the following expectations in the absence of nonallelic interactions (Table 2).

In the absence of both correlated gene distributions and nonallelic interactions the variances of arrays are homogeneous.

In the presence of nonallelic interactions contributions from the *l* and *j* type interactions contribute to the heterogeneity of the array variances, which have a mean value of $\Sigma u_a v_a h_a^2 + u_b v_b h_b^2 + 2(\alpha\delta\text{-}\beta\gamma)(\alpha+\delta\text{-}\beta\text{-}\gamma)\, h_a h_b + [\alpha\beta(1+v_a) + \gamma\delta(1+u_a)]\, j_{a/b}^2 + [\alpha\gamma(1+v_b) + \beta\gamma(1+u_b)]\, j_{b/a}^2 + [2\alpha\delta(\beta+\gamma) - 2\beta\gamma(\alpha+\delta)]\, j_{a/b} \cdot j_{b/a} + 2(\alpha\gamma\text{-}\beta\delta)\ h_a \cdot j_{b/a} + 2(\alpha\beta\text{-}\gamma\delta)\ h_b \cdot j_{a/b} + \tfrac{1}{4}(\alpha+\delta)(\beta+\gamma)\ l_{ab}^2 + (\alpha\gamma\text{-}\beta\delta)(\beta+\delta\text{-}\alpha\text{-}\gamma)\ h_a \cdot l_{ab} + (\alpha\beta\text{-}\gamma\delta)(\gamma+\delta\text{-}\alpha\text{-}\beta)\ h_b \cdot l_{ab} + 4[\alpha\gamma(\delta\text{-}\beta) + \beta\delta(\gamma\text{-}\alpha)]\ h_a \cdot j_{a/b} + 4[\alpha\beta(\delta\text{-}\gamma) + \gamma\delta(\beta\text{-}\alpha)]\ h_b \cdot j_{b/a} + (\gamma\delta\text{-}\alpha\beta)\ j_{a/b} \cdot l_{ab} + (\beta\delta\text{-}\alpha\gamma)\ j_{b/a} \cdot l_{ab}$.

Obviously, in the presence of nonallelic interactions the dominance contributions are inseparable from those of the interactions. While, however, the primary object in estimating this component is thereby lost, it may nevertheless have some predictive properties.

TABLE 2

Analysis of the dominance components in the absence of nonallelic interactions

Statistic	Expectations: Correlated gene distributions	Expectations: Random gene distribution	Symbol
Mean	$\Sigma u_a v_a h_a$	$\Sigma u_a v_a h_a$	$[\bar{h}]$
Mean variance of arrays	$\Sigma u_a v_a h_a^2 + u_b v_b h_b^2 + 2(\alpha\delta-\beta\gamma)(\alpha+\delta-\beta-\gamma)h_a h_b$	$\Sigma u_a v_a h_a^2$	$\frac{1}{4}H_1$
Variance of array means	$\Sigma u_a v_a h_a^2 + u_b v_b h_b^2 - 4u_a^2 v_a^2 h_a^2 - 4u_b^2 v_b^2 h_b^2 + [2\alpha\delta+2\beta\gamma + 4\beta\gamma(\alpha+\delta) + 4\alpha\delta(\beta+\gamma) - 8u_a u_b v_a v_b]h_a h_b$	$\Sigma u_a v_a h_a^2 - 4u_a^2 v_a^2 h_a^2$	$\frac{1}{4}H_1 - \frac{1}{4}H_2$
Total variation	$\Sigma 2u_a v_a h_a^2 + 2u_b v_b h_b^2 - 4u_a^2 v_a^2 h_a^2 - 4u_b^2 v_b^2 h_b^2 + [4(\alpha\delta+\beta\gamma) - 8u_a u_b v_a v_b]h_a h_b$	$\Sigma 2u_a v_a h_a^2 - 4u_a^2 v_a^2 h_a^2$	$\frac{1}{2}H_1 - \frac{1}{4}H_2$
$H_1 = 4\Sigma u_a v_a h_a^2 + u_b v_b h_b^2 + 2(\alpha\delta-\beta\gamma)(\alpha+\delta-\beta-\gamma)h_a h_b =$		$4\Sigma u_a v_a h_a^2$	
$H_2 = 16\Sigma u_a^2 v_a^2 h_a^2 + u_b^2 v_b^2 h_b^2 + [\frac{1}{2}u_a u_b v_a v_b - \frac{1}{2}\alpha\delta(\beta+\gamma) - \frac{1}{2}\beta\gamma(\alpha+\delta)]h_a h_b =$		$16\Sigma u_a^2 v_a^2 h_a^2$	

TABLE 3

Analysis of the i *type interactions*

Statistic	Expectations: Correlated gene distribution	Random gene distribution	Symbols
Mean	$\Sigma 2(\alpha\delta-\beta\gamma)i_{ab}$	0	$[\bar{i}]$
Mean variance of arrays	$\Sigma[2(\alpha\delta+\beta\gamma) - \alpha\delta(\alpha+\delta) - \beta\gamma(\beta+\gamma)]i_{ab}^2$	$\Sigma 3u_a u_b v_a v_b i_{ab}^2 = \frac{3}{16}I$	
Variance of array means	$\Sigma[\alpha\delta(\alpha+\delta) + \beta\gamma(\beta+\gamma) - 4(\alpha\delta-\beta\gamma)]i_{ab}^2$	$\Sigma u_a u_b v_a v_b i_{ab}^2 = \frac{1}{16}I$	
Total variation	$\Sigma[2(\alpha\delta+\beta\gamma) - 4(\alpha\delta-\beta\gamma)^2]i_{ab}^2$	$\Sigma 4u_a u_b v_a v_b i_{ab}^2 = \frac{1}{4}I$	

The interaction components

The expectations for the analysis of a derived diallel table containing the estimates of the i type interaction component of the generation means of each cross are given in Table 3. These expectations are the heritable components of variation for any number of independent digenic interactions.

The coefficients of the symbols in the last column have been chosen such that when $u_a = u_b = v_a = v_b$ etc. $I = \Sigma i_{ab}^2$, they do not, of course, hold for the expectations with correlated gene distributions.

The mean value of $[i]$, over all the crosses of a diallel set, has a value of zero only if the interacting genes are randomly distributed. A significant value for $[i]$, therefore, is indicative of correlated gene distributions.

The expectation for the analysis of the j type interactions, which are listed below (Table 4), are similar to those given in Table 3 for the I interactions except of course we are dealing with the sum or balance of effects due to both $j_{a/b}$ and $j_{b/a}$ terms.

As in the case of dominance we can recognize two components for the l type interactions which we will designate L_1 and L_2, being strictly analogous to H_1 and H_2. With random gene distributions $\frac{\frac{1}{4} H_1}{H_2} = \overline{uv}$ (JINKS 1954). Similarly $\frac{\frac{1}{16} L_2}{L_1}$ $= \overline{u_a u_b v_a v_b}$ which has an upper limit of $\frac{1}{16}$ when $u_a = u_b = v_a = v_b \ldots$ etc. $= \frac{1}{2}$ for the genes showing the l type interactions. The other expectations for this heritable component are as follows.

Interpretation with random gene distributions

With random gene distributions and no nonallelic interactions we estimate three genetic parameters D, H_1 and H_2 as originally defined by JINKS and HAYMAN (1953) from which we can derive the dominance ratio $\frac{H_1}{D}$ and $\overline{uv}$. Homogeneity of array variances for the additive component confirms the absence of j type nonallelic interactions and the homogeneity of array variances for the dominance component, the absence of both correlated gene distributions for the genes showing dominance and j and l type allelic interactions.

With random gene distributions and nonallelic interactions present D, H_1 and H_2 have no simple genetical interpretation and the estimates of D and H_1 will be heterogeneous over arrays. Estimates of I, J and L_1 will be directly comparable, having similar coefficients, and these estimates will be homogeneous over the various statistics available for their estimation.

Interpretation with non random gene distributions

In the absence of nonallelic interactions we can again estimate D, H_1 and H_2 but while our estimate of D will be homogeneous over arrays that of H_1 will not. The dominance ratio becomes

$$\frac{H_1}{D} = \frac{\Sigma u_a v_a h_a^2 + u_b v_b h_b^2 + 2(\alpha\delta - \beta\gamma)(\alpha + \delta - \gamma - \beta) h_a h_b}{\Sigma u_a v_a d_a^2 + u_b v_b d_b^2 + (\alpha\delta - \beta\gamma) d_a d_b}$$ which, of course,

TABLE 4

Analysis of the j *type interactions*

Statistic	Expectations: Correlated gene distribution	Expectations: Random gene distribution	Symbols
Mean	0	0	$[\bar{j}]$
Mean variance of arrays	$\Sigma\alpha\delta(2-\alpha-\delta)(j_{a/b}+j_{b/a})^2+\beta\gamma(2-\beta-\gamma)(j_{a/b}-j_{b/a})^2$	$\Sigma 3u_au_bv_av_b(j_{a/b}{}^2+j_{b/a}{}^2)$	$=\frac{3}{16}J$
Variance of array means	$\Sigma\alpha\delta(\alpha+\delta)(j_{a/b}+j_{b/a})^2+\beta\gamma(\beta+\gamma)(j_{a/b}-j_{b/a})^2$	$\Sigma u_au_bv_av_b(j_{a/b}{}^2+j_{b/a}{}^2)$	$=\frac{1}{16}J$
Total variation	$\Sigma 2\alpha\delta(j_{a/b}+j_{b/a})^2+2\beta\gamma(j_{a/b}-j_{b/a})^2$	$\Sigma 4u_au_bv_av_b(j_{a/b}{}^2+j_{b/a}{}^2)$	$=\frac{1}{4}J$

TABLE 5

Analysis of the l *type interactions*

Statistic	Expectations: Correlated gene distributions	Expectations: Random gene distributions	Symbols
Mean	$\Sigma 2(\alpha\delta+\beta\gamma)l_{ab}$	$\Sigma 4u_au_bv_av_bl_{ab}$	$[\bar{l}]$
Mean variance of arrays	$\Sigma[2(\alpha\delta+\beta\gamma)-\alpha\delta(\alpha+\delta)-\beta\gamma(\beta+\gamma)]l_{ab}{}^2$	$\Sigma 3u_au_bv_av_bl_{ab}{}^2$	$\frac{3}{16}L_1$
Variance of array means	$\Sigma[\alpha\delta(\alpha+\delta)+\beta\gamma(\beta+\gamma)-4(\alpha\delta+\beta\gamma)^2]l_{ab}{}^2$	$\Sigma u_au_bv_av_bl_{ab}{}^2-16u_a{}^2u_b{}^2v_a{}^2v_b{}^2l_{ab}{}^2$	$\frac{1}{16}L_1-\frac{1}{16}L_2$
Total variation	$\Sigma[2(\alpha\delta+\beta\gamma)-4(\alpha\delta+\beta\gamma)^2]l_{ab}{}^2$	$\Sigma 4u_au_bv_av_bl_{ab}{}^2-16u_a{}^2u_b{}^2v_a{}^2v_b{}^2l_{ab}{}^2$	$\frac{1}{4}L_1-\frac{1}{16}L_2$

$L_1=\Sigma_3^{16}[2(\alpha\delta+\beta\gamma)-\alpha\delta(\alpha+\delta)-\beta\gamma(\beta+\gamma)]l_{ab}{}^2=$ $\Sigma 16u_au_bv_av_bl_{ab}{}^2$

$L_2=\Sigma\frac{1}{3}[32(\alpha\delta+\beta\gamma)+192(\alpha\delta+\beta\gamma)^2-64\alpha\delta(\alpha+\delta)-64\beta\gamma(\beta+\gamma)]l_{ab}{}^2=$ $\Sigma 256u_a{}^2u_b{}^2v_a{}^2v_b{}^2l_{ab}{}^2$

may be greater or less than $\frac{\Sigma uvh^2}{\Sigma uvd^2}$ according to the sign of the correlation between the gene distributions (HAYMAN 1954; JINKS 1955, 1956).

This estimate of the dominance ratio however, is only seriously upset by gene distributions which are highly negatively correlated in the parental lines.

In the presence of nonallelic interactions the estimates of I, J and L, obtained from the mean variance of arrays will still be directly comparable but the estimates of I and J obtained from the mean variance of arrays and the variance of array means will no longer be homogeneous. Or to put it another way, the heritable component of the mean variance of arrays is no longer three times that of the variance of array means for the I and J components of variation. For example in the extreme case of complete association the mean variance of arrays for the i interactions reduces to $\alpha\delta\Sigma i_{ab}{}^2$ and the variance of array means to $\Sigma\alpha\delta(1-4\alpha\delta)i_{ab}{}^2$, therefore the ratio of the former to the latter tends to ∞ as $\alpha\delta$ tends to its maximum value of $\frac{1}{4}$. On the other hand, for the j interactions complete association would lead to expectations for the heritable component of both the mean variance of arrays and variance of array means equal to $\Sigma\alpha\delta(j_{a/b} + j_{b/a})^2$, thus giving a ratio of one.

A ratio of less than one for the j type interactions and greater than three for the i type interactions is, therefore, indicative of correlated distributions of the interacting genes.

Interpretation with linkage

Linkage only affects the nonallelic interaction terms in the generation means but in the presence of nonallelic interactions it affects all the components of these means using the method of estimation described here. In the absence of nonallelic interactions, therefore, the interpretation of the statistics given earlier still stands even in the presence of linkage. In the presence of nonallelic interactions, however, even with information from more generations than used here, we could not obtain estimates of D, H_1, H_2, I, L_1 and L_2 unconfounded with the effects of linkage. Hence the complications of linkage like those due to correlated gene distributions in the parental lines, may be detected but not estimated independently from the effects due to nonallelic interactions.

Example

Illustrations are taken from the diallel sets of crosses between eight inbred varieties of *N. rustica* (JINKS 1954, 1956) the character chosen being plant height. The data consists of the family means of the parents F_1, F_2 and backcross generations of 28 crosses (averaged over reciprocals) grown in two blocks in each of two seasons (JINKS 1954, 1956). For each cross in each block we can estimate the value of $[d]$, $[h]$, $[i]$, $[j]$ and $[l]$ (JINKS and MORLEY JONES 1958). Thus for each of the components of the generation means we have four derived diallel tables giving 111 degrees of freedom in all. Of these, 27 are for comparisons between crosses, one for between seasons and two between blocks, leaving 27 and

54 for the two interaction sums of squares respectively. These analyses are given below

Analysis of the components of the family means

Components Item	N	MS	$\frac{[d]}{V_R}$	P	MS	$\frac{[h]}{V_R}$	P	MS	$\frac{[i]}{V_R}$	P	MS	$\frac{[j]}{V_R}$	P	MS	$\frac{[l]}{V_R}$	P
Crosses	27	44·38	2·00	·05–·01	421·78	2·99	·001	227·61	1·78	0·05	52·93	3·12	·01–·001	576·73	1·75	·05
Seasons	1	12·29	...	....	22·05	...	....	145·93	...	...	11·93	...	....	4503·43	13·66	·001*
Blocks	2	7·73	....	....	37·11	...	....	45·23	...	...	10·25	...	....	1418·57	4·30	·05–·01
Crosses × Seasons	27	26·84	1·21	....	224·55	1·59	·05–·01	210·38	1·64	0·05	58·73	3·46	·01–·001	660·35	2·00	·05–·01
Crosses × Blocks	54	22·20	...	....	141·29	...	....	128·21	...	...	16·99	...	....	329·58	...	....

* P = 0·05–0·01 when tested against the crosses × seasons M.S.

All the heritable components of the family means make a significant contribution to the variation between crosses ($< 0{\cdot}05$) but for the components of nonallelic interactions the mean sums of squares for seasonal interactions are of the same order of magnitude as those due to differences between crosses. This, however, is not true of the additive and dominance components although the latter shows significant seasonal interactions.

The total variation between crosses for each of the heritable components can be partitioned into the variation within and between arrays from which we can derive the mean variance of arrays and the variance of array means respectively.

Since there is significant crosses × seasons interaction, for all but $[d]$ of the five components of the family means, these variances have been estimated independently for each season. Their nonheritable component (E_2) is then derived from the heterogeneity of crosses over blocks within each season. After subtracting this nonheritable portion of the variances we are left with the heritable components of the mean variances of arrays and variances or array means given in Table 6.

Only three of the above heritable components are nonsignificant at $P < 0{\cdot}05$. These are the mean variance of arrays for $[h]$, $[i]$ and $[l]$ in 1953.

TABLE 6

The heritable components of:—

Component	Season	Mean variance of arrays	Variance of array means
$[d]$	1952	4·48	3·64
	1953	5·19	7·01
$[h]$	1952	112·13	23·93
	1953	52·99	21·51
$[i]$	1952	73·05	8·01
	1953	0·95	3·85
$[j]$	1952	11·33	4·12
	1953	8·22	5·01
$[l]$	1952	160·24	42·67
	1953	58·96	23·28

There are a number of predictions we can test on the heritable components of these variances. Firstly, since there are significant j type interactions we would expect our estimates of D to be heterogeneous over arrays. A Bartlett test of homogeneity of variance on the eight array variances in each season showed no significant heterogeneity ($P > 0.20$). For the same reasons we would expect our estimates of D, obtained from the mean variance of arrays and the variance of array means, to differ. This expectation was also not fulfilled when tested by the method of COCHRAN (1951). Clearly any heterogeneity in the estimates of D produced by the j type interactions is swamped by other sources of variation.

A second prediction is that the array variances of the dominance component will be heterogeneous in the presence of significant J and L interactions. Since, however, neither L nor H_1 are significant in 1953 we can only expect to find significant heterogeneity in 1952. This is in fact observed. In 1952 Bartletts test gives a probability less than 0.001 while in 1953 the array variances are homogeneous ($P > 0.20$).

With random gene distributions the heritable portions of the mean variance of arrays for $[i]$ and $[j]$ should be three times those of the variance of array means being $\frac{3}{16}I$ and $\frac{3}{16}J$ and $\frac{3}{8}I$ and $\frac{3}{8}J$ respectively. Of the four available tests of these expectations, made by the method of COCHRAN (1951), only one, the ratio for $[i]$ for 1952 is significantly different from 3 with a probability of 0.05. The ratio for $[j]$ for both seasons is lower than expected for random gene distributions but are not significantly so in either season, while the numerator of this ratio for $[i]$ in 1953 is not significantly different from zero. While, therefore, there are some suggestions of deviations from random gene distributions for the interacting genes, these deviations are of the same order of magnitude as the heterogeneities over replicate blocks within the two seasons.

In the absence of significant deviations from random gene distributions our estimates of I and J are homogeneous over the mean variance of arrays and the variance of array means. We can, therefore, estimate these two heritable components of variation from the contributions of $[i]$ and $[j]$ to the total variation, which equal $\frac{1}{4}I$ and $\frac{1}{4}J$ respectively. These estimates along with those for D, H_1, H_2, L_1 and L_2 are given below in Table 7.

The significance of the heritable portion of the variance of array means for $[l]$

TABLE 7

The components of variation

Component	Season 1952	Season 1953
D	16·21	25·28
H_1	448·52	211·28
H_2	352·80	125·93
I	288·10	15·61
J	73·23	48·84
L_1	854·61	314·47
L_2	171·83	—57·95

in both seasons show that $(L_1 - L_2)$ does not equal zero, so $L_1 > L_2$. This in turn means that there are unequal gene frequencies for the interacting genes. Similarly, $H_1 > H_2$ in both seasons, although in the presence of significant nonallelic interactions the evidence for unequal gene frequencies for the genes showing dominance is ambiguous. Assuming random gene distributions we can solve for L_1 and L_2 giving the results shown in Table 7. A negative value for L_2 in 1953 is, of course, impossible and must reflect the correlation between the estimates of L_1 and L_2.

We can now compare the relative magnitudes of the heritable components of variation using once more the method of COCHRAN. Before doing so, however, we must remember that the estimates of the $[d]$'s and $[j]$'s, on the one hand, and those of the $[h]$'s, $[i]$'s and $[l]$'s on the other are correlated and that these correlations may well affect the relative magnitudes of the D's and J's or the H's, I's and L's estimated from them. We must, therefore, accept the following comparisons with reserve until such times as the effects of these correlations have been elucidated.

Assuming no correlated gene distribution the three nonallelic interaction components of variation, I, J and L_1 are directly comparable. If, however, there is any suggestion of correlated gene distributions we can still compare the heritable components of the mean variance of arrays for $[i]$, $[j]$ and $[l]$. In our example the result is the same on either test. Thus in 1952 I is not significantly different from L_1 but both are significantly greater than J while in 1953 there are no significant differences between any of the three.

In 1952 H_1 is significantly different from D and J but not from I or L, while in 1953 there are no significant differences.

One further expectation can be tested, namely, on the mean value of $[i]$ over all crosses of a diallel set i.e. $[\bar{i}]$ is zero with random distributions of the interacting genes. Using the heterogeneity over blocks within seasons as the error variance we find no significant deviation from this expectation ($P\ 0{\cdot}6 - 0{\cdot}7$).

CONCLUSIONS

The joint analysis of parents, F_1, F_2 and backcross generations of a diallel set of crosses presented here offers an alternative approach to the problem of estimating the components of variation to that described by JINKS (1956). The new method consolidates many of the individual tests of the earlier analyses into a single process of estimation of the contributions due to additive, dominance and nonallelic interaction components of variation. Tests for the presence of correlated gene distributions are provided by the heterogeneity of the estimate of two of these components, namely I and J.

In the absence of nonallelic interactions the two approaches give similar information about the additive and dominance components of variation. Correlated gene distributions are detected by curvature of the Wr/Vr regression in the one analysis and by heterogeneity of the dominance component over arrays in the other.

In the earlier analyses the effects of nonallelic interactions could be detected but not estimated by the Wr/Vr regression, the scaling tests and the heterogeneity of the additive and dominance components of variation over generations. In the new analysis the effects of nonallelic interactions are estimated and partitioned between four components I, J, L_1 and L_2. The presence of nonallelic interactions may also be indicated by the heterogeneity of the additive component over the statistics available for its estimation.

No method of diallel analysis allows one to estimate the additive and dominance components of variation unconfounded with contributions from nonallelic interactions when the latter are present. Nevertheless, these components still have some predictive value if only for the supplementary tests they provide for the presences of nonallelic interactions. In contrast our new approach does allow one to estimate the nonallelic interaction components of variation which are not confounded with additive or dominance effects.

The curvature of the Wr/Vr regression due to correlated gene distributions is difficult to detect in the presence of the upsets produced by nonallelic interactions. Our new method, however, provides tests of correlated gene distributions in the presence of nonallelic interactions based on the heterogeneity of the estimates of the I and J components of variation.

Since we are analysing generation means or the components of these means we cannot obtain conclusive evidence of linkage although, of course, linkage of interacting genes can effect these means. Previously linkage has been detected by including the variances of segregating F_2 and backcross generations in the analyses and this still remains the best approach with the present experimental design. But in this test, as in all others available, linkage is inseparable from correlated gene distributions in the parental lines. In the absence of nonallelic interactions linkage will pass undetected and it does not effect the estimated components D, H_1 and H_2. It will, however, affect their prospective predictive value.

The data used as illustrative material for the new analysis have been extensively analysed by the earlier methods and there are no disagreements between the conclusions obtained. For the first time, however, we have estimates of the magnitude of the nonallelic interaction components of variation and we have further shown that correlated gene distributions (or linkage) among the interacting genes is not an important source of variation in this data. In addition we have obtained confirmation of what was long suspected, namely, the importance of seasonal differences in the nonallelic interaction components of variation.

Clearly, where our interest is primarily in the components of variation this new approach has advantages over the earlier ones and its present form is readily adapted for use with fewer or more generations than those used here. When, however, our interest is centered more on the relative performance of crosses, or the distribution of dominant, recessive or interacting genes in the parental lines the earlier analysis depending on the Wr/Vr regression and scaling tests have the advantage.

SUMMARY

An alternative approach to the joint analysis of parental, F_1, F_2 and backcross generation means from a set of diallel crosses is presented. It is based on the analysis of variance of the components of these generation means and leads to the estimation of seven heritable components of variation, namely, D, H_1, H_2, I, J, L_1 and L_2.

Deviations from random gene distributions in the parental lines and linkage of interacting genes are detected by the heterogeneity of the estimates of I and J. The interpretation of the components in the presence of such disturbances is discussed.

Illustration of the new method with data from an 8×8 diallel set of crosses between inbred varieties of *N. rustica* has given results which are in good agreement with those of earlier analysis. In addition it has allowed the estimation and partitioning of the effects due to non-allelic interactions as well as a demonstration of their variability over seasons.

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TRANSDUCTION OF THE GALACTOSE REGION OF ESCHERICHIA COLI K12 BY THE PHAGES λ AND λ-434 HYBRID

ALLAN CAMPBELL AND EVELYN BALBINDER

Carnegie Institution of Washington, Department of Genetics
Cold Spring Harbor, Long Island, New York

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JACOB and WOLLMAN (1957) have isolated from nature a number of bacteriophages which are of interest because they are serologically related to the well-studied phage λ and can be crossed with it. Each of these differs from λ at many loci, and in particular in a region near the center of the chromosome (c_I region) which determines the immune specificity of the phage (KAISER and JACOB 1957). These phages are, in BERTANI's (1958) terminology, *dismune* from each other and from λ. A lysogenic bacterial strain will be lysed by the external addition of a temperate phage related to the prophage it carries if the two phages are dismune, but not if they are *coimmune*. It has also been shown (JACOB and WOLLMAN 1957) that each of these phages has a specific point of attachment onto the bacterial chromosome, and that in some cases this specificity is determined by the c_I region.

By a series of backcrosses with λ, KAISER and JACOB (1957) have obtained a strain called the λ-434 hybrid, which should be isogenic with λ except for the c_I region. The present report will deal with a comparison of the properties of this hybrid strain with those of phage λ in transductions of the galactose region. It will be convenient for our purposes to consider the λ and 434 c_I regions as alleles, designated by imm^{λ} and imm^{434} respectively. The λ-434 hybrid will then be denoted by λimm^{434}.

Previous work (CAMPBELL 1957; ARBER *et al.* 1957) has indicated that transductions of the galactose region by lambda are performed not by the phage itself, but by a new type of genetic structure which has been formed by recombination between phage and host material. This structure contains some of the genetic material of the phage, including the c_I region, but lacks a portion of it, which contains the genes *h* and m_s. Instead of this missing piece, it contains a region of host genetic material including some genes concerned with galactose fermentation.

When a sensitive cell of *Escherichia coli* K12 is singly infected with a mature transducing particle, lysogenization may occur. The resulting cell is immune to superinfection by phages of the same immune specificity, but it is not a true lysogen. Presumably because of the large "deletion" of phage genetic material, a complete cycle of vegetative multiplication and maturation does not follow induction in such cells. These strains have therefore the standard properties of inducible defective lysogens (JACOB and WOLLMAN 1957). The lysogeny of these cells is slightly unstable, the "prophage" being lost about once every 10^3 cell divisions. The resulting substrain will be haploid for the gal region, and with

appropriately marked stocks may frequently be detectable as a *gal*$^-$ segregant from a *gal*$^+$ strain.

If the recipient is lysogenic, or if a sensitive recipient is doubly infected by a transducing particle and an active phage, a double lysogen carrying one active and one defective (transducing) prophage usually results. Induction of such cells yields lysates containing a mixture of mature active phages and transducing particles. As in the defective lysogen, the transducing prophage is occasionally lost, as detected by the change from *gal*$^+$ to *gal*$^-$ in appropriately marked stocks. These single and double lysogens have been called *immune syngenotes* and *lysogenic syngenotes* respectively (Morse *et al.* 1956b). If the gal region in the transducing prophage is genetically different from that in the bacterial chromosome, the cell is a *heterogenote;* if the two are identical, it is a *homogenote.*

In some of the experiments we will report here in which more than one phage is used, the outcome depends on whether or not the two are coimmune. For others, immune specificity is an irrelevant variable and can be treated simply as a genetic marker of the phage. We have in some cases verified that the latter is true by comparing an experiment using λimm^{λ} and λimm^{434} with the same experiment using $\lambda co_2{+}imm^{\lambda}$ and $\lambda co_2 imm^{\lambda}$, where co_2 is a gene located close to the c_I region. *Terminology:* A defective phage is not a phage. It does not satisfy the accepted definition of a virus (Luria 1953). Unfortunately, there is no good generic term which includes both phage and defective phage in all stages of their life cycles and excludes other entities such as transforming principles and bacterial genes. Such a definition cannot be satisfactorily framed until we understand more about the mode of attachment of the prophage to the bacterial chromosome. Therefore we will adopt the temporary expedient of including both defective and active phages under the generic term *phage*, (Arber *et al.* 1957). If we describe a strain as, say, triply lysogenic, we mean that the total number of carried prophages, active and defective, is three.

The standard recipient strain we have used is $gal_1{}^-gal_2{}^-$. Since we shall generally not have occasion to distinguish these genes individually, we will denote this state of the gal region simply by *gal*$^-$. A transducing particle carrying particular alleles (either in the mature or prophage state) will be characterized by symbols such as $\lambda gal{+}co_2{+}imm^{434}$, or $\lambda gal{+}co_2 imm^{\lambda}$. The order of listing these genes carries no implications concerning their order on the genetic map of the transducing particle, this map being at present unknown.

Thus a strain which has been derived by transducing $gal_1{}^-gal_2{}^-$ cells carrying the prophage $\lambda m_5 co_2 imm^{\lambda}$ with the lysate from a lysogenic heterogenote the active phage from which are all $m_5{+}co_2{+}imm^{\lambda}$ can be given the symbol K12 *gal*$^-$ ($\lambda m_5 co_2$ imm^{λ}) ($\lambda gal{+}co_2{+}imm^{\lambda}$). The allele $m_5{+}$ is not included because it is located in the "deleted" piece of the defective phage, as verified by the fact that the active phage liberated by the double lysogen here derived are all genetically m_5. Because the defective and active phages can recombine, presumably in the vegetative state (Arber *et al.* 1957) some of the mature active phage produced will be $m_5 co_2{+}$ imm^{λ} and others $m_5 co_2 imm^{\lambda}$.

Such notations are always tentative and represent the simplest interpretation of the data. The primary observations can better be symbolized K12 ($m_5, co_2^+/co_2$, imm^λ, gal^+/gal^-). We will try to use both notations in a manner which we hope distinguishes observations from inferences without obscuring the logic of the experiments in terms of our working model.

In a previous publication (CAMPBELL 1957) we defined the *efficiency of transduction* as the number of transductions observed per plaque-forming particle adsorbed. Since the transductions are not performed by plaque-forming particles this was not a wise nomenclature, and we will use here instead the term transductions per phage (T/P) (ZINDER 1955).

MATERIALS AND METHODS

The bacteria used are all derivatives of *Escherichia coli* K12, and the phage are reference type λ and various mutants thereof. Their origin has been previously related (CAMPBELL 1957). The λ-434 hybrid was kindly supplied by DR. A. D. KAISER.

Transductions were performed by adding appropriate amounts of phage to a suspension of bacteria in 0.01 M $MgSO_4$. The bacteria were previously grown in aerated broth to a density of about 10^9 and then spun and resuspended at one third their original concentration. After 20 minutes of adsorption, the mixture of phage and bacteria was diluted into anti-λ serum in broth and incubated for thirty minutes before plating.

Plating was regularly performed on minimal agar (M) without glucose (OZEKI 1956) containing 0.4 percent galactose and 0.01 percent 2,3,5,-triphenyl-2H-tetrazolium chloride (EASTMAN). Plates were incubated five days, after which no further increase in number of gal^+ colonies was observed. On this indicator, gal^+ colonies appear dark red and gal^- colonies are white.

All segregation patterns described here were performed on /λ mutants of the strains examined. They were isolated by selection against a strong virulent mutant of λ. Segregants were tested for carried phage by plating supernates of chloroformed cultures on C600. Immunity was determined by spot testing on K12 (λimm^λ) and K12 (λimm^{434}).

EXPERIMENTAL

I. *Low frequency transduction by the hybrid phage:* If sensitive K12 gal^- bacteria are infected with a lysate produced by ultraviolet induction of K12 gal^+ lysogenic for λimm^λ, a small number of the survivors are gal^+, and most of these are lysogenic heterogenotes. (MORSE *et al.* 1956a). This is called *low frequency transduction* (LFT) as contrasted with the *high frequency transduction* (HFT) manifested by lysates of syngenotes. We know virtually nothing at present about the mechanism of low frequency transduction. It is of interest to know whether LFT is possible with lysates from K12 gal^+ carrying prophage with the imm^{434} marker, which is located at a different point on the bacterial chromosome.

TABLE 1

Low frequency transductions by λimm^{434} *and* λimm$^{\lambda}$

Bacterial donor	T/P assayed on K12 *gal*$^-$	Characteristics of *gal*$^+$ transduction clones			
		Lysogenic	Heterogenotic	Stable	HFT lysate produced on induction
C600 *gal*$^+$ (λ*imm*^{434})					
Strain 1*	4.6×10^{-7}†	4/4	4/4	0/4	4/4
Strain 2*	6.9×10^{-8}†				
C600 *gal*$^+$ (λ*imm*$^{\lambda}$)	1.6×10^{-6}†	4/4	3/4	1/4	Not tested

* Strains 1 and 2 are independently lysogenized substrains of C600.
† Multiplicity of infection: 2 to 3.

TABLE 2

High frequency transductions by λimm^{434}

Bacterial recipient	Bacterial donor*	*Gal*$^+$ transduction clones†				
		Lysogenic			Defective	Sensitive
		h imm^{434}	*h*$^+$ *imm*^{434}	*h imm*$^{\lambda}$/*imm*^{434}	*imm*^{434}	
K12 *gal*$^-$(λ*h imm*$^{\lambda}$)	A	0	0	7	2	0
	B	0	0	7	2	0
	C	2	0	7	1	0
K12 *gal*$^-$	A	0	2	0	25‡	7
Nonlysogenic	C	0	1	0	12‡	9

Transductions by HFT lysates from lysogenic heterogenotes derived by transduction with λ*h*$^+$*imm*^{434} LFT.
* Strains A, B, and C are lysogenic heterogenotes produced by transducing *gal*$^+$ into K12 *gal*$^-$ with λ*h*$^+$*imm*^{434} LFT.
† Multiplicity of infection: 5×10^{-8}.
‡ One *gal*$^-$ substrain from each of 19 of these 37 defective heterogenotes was tested. All were sensitive to λ.

The data of Tables 1 and 2 show that it is, and furthermore that the properties of the heterogenotes produced are very similar to those which have previously been studied with phage λ*imm*$^{\lambda}$. Lysates from such heterogenotes contain a mixture of active and defective phages, and these defective phages carry no copy of the *h* gene. Thus only two of the transduction clones of Table 2 carry the allele *h*$^+$, although all of them which are not sensitive have acquired the allele *imm*^{434} from the transducing lysate.

Transducing particles with the 434 immune specificity can also be derived by recombination between *imm*^{434} active phage and *gal*$^+$*imm*$^{\lambda}$ defectives. For example, if K12 *gal*$^-$ (λ*imm*^{434}) is infected with the lysate produced by induction of K12 *gal*$^-$ (λ*imm*$^{\lambda}$) (λ*gal*$^+$*imm*$^{\lambda}$), some of the resulting *gal*$^+$ strains are lysogenic heterogenotes carrying only the 434 immune specificity. These have the presumed structure K12 *gal*$^-$(λ*imm*^{434}) (λ*gal*$^+$*imm*^{434}). No difference has been observed between such heterogenotes and those described in Tables 1 and 2.

High frequency transduction using lysates from heterogenotes with different immune specificities: We have previously reported that the number of transductions per phage produced when sensitive *gal*$^-$ cells are infected with the lysate

from a lysogenic heterogenote increases with increasing multiplicity because the probability that a transducing particle will lysogenize is greatly increased by simultaneous infection with an active phage (CAMPBELL 1957). A similar effect on frequency of lysogenization has been observed by JACOB *et al.* (1957) with certain defective phages not isolated for their transducing ability. The mechanism is not thoroughly understood. ARBER *et al.* (1957) have shown that some of the cells singly infected with transducing particles lyse, but quantitatively it is not clear whether we are dealing solely with a prevention of lysis. If simultaneous infection with a nontransducing lysate increases the number of recovered transductions above that found when a transducing lysate is used alone, we shall say that the phage from the nontransducing lysate *helps*. We can then examine various situations to see when such help occurs.

We also know from previous work that there is no helping effect if a lysogenic recipient is used, when all phages involved have the λ immune specificity. The number of transductions per phage is in this case constant over the range of multiplicities studied and is approximately equal to that observed with sensitive recipients at low multiplicities. This result might mean that the immunity of the lysogenic cell interferes with the helping action, especially in view of the fact that helping is regularly accompanied by lysogenization of the cell by the helping phage. We expect the prophage to interfere with lysogenization by a coimmune phage, but we do not know whether lysogenization by the helping phage is a necessary or an incidental concomitant of the helping effect. Since the helping action involves two particles (helping phage and transducing particle), one can gain more insight by examining what happens when the two have different immune specificities.

Table 3 shows the result of a typical experiment in which the helping phage, the prophage, and the transducing particle were in turn varied over the domain imm^{λ}, imm^{434}, and absent. The third case is trivial for the transducing particle. It is omitted from the table but was included in the experiments as a control in which no transductions were ever observed.

TABLE 3

Transduction by λimm$^{\lambda}$ *and* λimm^{434} *in single and mixed infection*

Bacterial recipient	Transducing lysate: λ*imm*$^{\lambda}$ Nontransducing lysate			Transducing lysate: λ*imm*^{434} Nontransducing lysate		
	None	λ*imm*$^{\lambda}$	λ*imm*^{434}	None	λ*imm*$^{\lambda}$	λ*imm*^{434}
K12	1.9×10^{-2}	2.9×10^{-1}	3.5×10^{-1}	3.2×10^{-3}	8.7×10^{-2}	4.7×10^{-2}
K12 (λ*imm*$^{\lambda}$)	1.6×10^{-2}	1.5×10^{-2}	5.2×10^{-2}	1.1×10^{-2}	8.4×10^{-3}	3.3×10^{-2}
K12 (λ*imm*^{434})	6.6×10^{-2}	2.4×10^{-1}	3.8×10^{-2}	4.5×10^{-3}	3.4×10^{-2}	3.6×10^{-3}

Numbers recorded are transductions per adsorbed phage. Multiplicities of infection:
Nontransducing: λ*imm*$^{\lambda}$, 6.5; λ*imm*^{434}, 3.1.
Transducing: λ*imm*$^{\lambda}$, 1.6×10^{-3}; λ*imm*^{434}, 1.6×10^{-3}.

On the basis of these data and those of similar experiments, the following two rules can be stated:

(1) Added nontransducing phage will always help unless the recipient cell carries a prophage which is coimmune with it.

(2) In the absence of added nontransducing phage, the number of transductions recovered is higher if the recipient carries a prophage of immune specificity different from that of the transducing particle than if it is sensitive or carries a prophage of the same immune specificity.

Rule (1) answers our question. A deeper understanding of the mechanism will be possible only when we know more about immunity in general.

TABLE 4

Transductions into lysogenic strains

Bacterial recipient	Bacterial donor	Gal⁺ transduction clones				
		Lysogenic			Defective	
		imm^{λ}/imm^{434}	imm^{λ}	imm^{434}	imm^{λ}	imm^{434}
K12 *gal*⁻(λimm^{λ})	K12 *gal*⁻(λimm^{434}) (λ *gal*⁺ imm^{434})	126	3	6	1	12
K12 *gal*⁻(λimm^{434})	K12 *gal*⁻(λimm^{λ}) (λ *gal*⁺ imm^{λ})	117	1	5	3	0

Pooled data from several experiments. The multiplicity of infection was always in the range 10^{-3} to 10^{-2}.

Genetic content of strains produced by transductions: Table 4 shows the genetic content and inferred structures of *gal*⁺ strains derived by infecting lysogenic *gal*⁻ recipients with high frequency transducing lysates at multiplicities less than 10^{-2}. These structures are deduced in part from the segregation data to be discussed in the next section. In all cases we see that the most common type of transduction clone is one which contains the *c* region from both donor and recipient.

There is one significant difference between these data and those of the third row of Table 6, in which the donor phage and the prophage have the same immune specificity but carry different alleles of another gene (co_2) which is closely linked to the c_I region. When the immune specificities of donor and recipient differ, about four percent of the *gal*⁺ strains produced by transduction are defective lysogens, usually with the immune specificity of the donor. These may be interpreted as prophage substitutions. When both donor and recipient carry the immune specificity imm^{λ}, no substitutions occur.

Segregation patterns of strains produced by transduction: When a strain of the type K12 *gal*⁻ (λimm^{434}) is rendered *gal*⁺ by infection with a lysate containing $\lambda gal^{+}imm^{\lambda}$ particles, one produces lysogenic heterogenotes exhibiting both immune specificities. From their origin, one would expect these strains to have the constitution K12 *gal*⁻ (λimm^{434}) ($\lambda gal^{+}imm^{\lambda}$). It is desirable to have some independent check that the genetic material of the two prophages actually does retain its original coupling relationship. As was pointed out by APPLEYARD (1954), one such check can be obtained by examining a number of substrains independently

derived from the double lysogen, each of which is altered in its net content of prophage alleles. The fact that a set of alleles is regularly lost from the cell as a unit constitutes evidence that they are organized into one structure within the cell.

Lysogens carrying the transducing prophage furnish excellent material for such studies because of the frequent and easily detectable loss of this prophage. We have mentioned earlier the correlated loss of the *gal*+ character and immunity from strains of the types K12 *gal*⁻ (λgal^+imm^λ) and K12 *gal*⁻ (λgal^+imm^{434}). By analogy, we might anticipate that the strains of the preceding paragraph which are expected to be K12 *gal*⁻ (imm^{434}) (λgal^+imm^λ) would exhibit a correlated loss cf the properties *gal*+ and imm^λ.

Table 5 shows the results of the analysis of ten double lysogens from each of the three groups. To prevent complications due to readsorption within the colonies, a λ-resistant mutant of each strain was first isolated by cross streaking against a strong virulent mutant of λ. Each *gal*⁻ derivative was selected from a different *gal*+ colony whose content of prophage alleles was identical with that of the parent strain.

All 30 strains show a strong tendency for loss of a copy of the c region to be correlated with loss of the *gal*+ character. Generally the c region lost is that which entered the cell in conjunction with *gal*+; so that the original coupling relationship is retained. Within each strain there are some individual exceptions, and in six of the 30 strains the coupling is reversed. These exceptions are all explainable in terms of various types of prophage recombination (APPLEYARD 1954).

Special comment should be made on those strains which are *gal*⁻ but retain both copies of the c region. In the case of the single lysogen K12 *gal*⁻ (λgal^+imm^λ), all *gal*⁻ derivatives which are still immune have been found to be K12 *gal*⁻ (λgal^-imm^λ); i.e., *homogenotes* (MORSE, personal communication). We have examined 13 independently derived *gal*⁻ segregants from the strain K12 gal_1^-(λimm^{434}) (λgal^+imm^λ) /λ which liberated phages of both immune specificities. Of these, six were gal_1^- homogenotes, but the other seven were haploids. The latter group must result from recombination between the two carried prophages.

Triple lysogens: We have previously performed the following experiment (CAMPBELL 1957). A culture of K12 *gal*⁻ ($\lambda m_5 co_2 imm^\lambda$) is infected with the lysate prepared by inducing the heterogenote K12 *gal*⁻ ($\lambda h\ m_5^+co_2^+imm^\lambda$) ($\lambda gal^+co_2^+imm^\lambda$). Most of the resulting *gal*+ colonies are of the type K12 (gal^+/gal^-, m_5, h^+, co_2/co_2^+, imm^λ) but a few are K12 (gal^+/gal^-, m_5^+/m_5, h/h^+, co_2/co_2^+, imm^λ). We referred to the latter as double lysogens but pointed out that they might very well carry three prophages. In that case we would now write their structure as K12 $gal^-(\lambda m_5^+hco_2^+imm^\lambda)$ $(\lambda m_5h^+co_2imm^\lambda)$ $(\lambda gal^+co_2^+imm^\lambda)$. They would result from simultaneous superinfection of the original lysogen by an active and a defective phage.

An alternative explanation was not fully ruled out by the previous data. Suppose that in the strain K12 ($\lambda h\ m_5^+co_2^+imm^\lambda$) ($\lambda gal^+co_2^+imm^\lambda$) there could arise by recombination some particles of the type $\lambda h\ m_5^+gal^+co_2^+imm^\lambda$. We do not

know whether phage with such a genome would be active or defective. If such a phage were to lysogenize K12 *gal*$^-$ ($\lambda m_s co_s imm^\lambda$), the resulting strain would be K12 *gal*$^-$ ($\lambda m_s h^+ co_s imm^\lambda$) ($\lambda m_s^+ hgal^+ co_s^+ imm^\lambda$), which would have the correct content of prophage alleles. Furthermore, a strong correlation in the loss of the "*gal*$^+$" and "*h*" characters was observed in these strains—a result not predictable for the expected triple lysogen.

To distinguish these alternatives, we have systematically varied the multiplicity of infection during transduction and have tested the *gal*$^+$ colonies produced

TABLE 5

Gal$^-$ *segregants from double lysogens*

Prophages carried by		Isolate	Alleles from *c* region present in *gal*$^-$ segregants			
Bacterial recipient	Bacterial donor		Both alleles	Allele from recipient only	Allele from donor only	Non lysogenic
$\lambda m_s co_s imm^\lambda$	$\lambda m_s^+ co_s^+ imm^\lambda$, λ *gal*$^+$ $co_s^+ imm^\lambda$	1	4	2	10	0
		2	1	5	11	0
		3	1	11	4	0
		4	3	10	5	0
		5	3	12	1	0
		6	1	15	2	0
		7	2	6	15	0
		8	2	16	6	0
		9	2	9	11	0
		10	2	20	2	0
λimm^{434}	λimm^λ, λ *gal*$^+$ imm^λ	11	2	21	4	0
		12	5	21	7	0
		13	2	12	3	0
		14	4	9	7	0
		15	2	17	2	0
		16	2	13	3	0
		17	4	18	0	0
		18	2	2	18	0
		19	0	13	5	0
		20	3	4	13	0
λimm^λ	λimm^{434}, *gal*$^+$ imm^{434}	21	2	15	4	0
		22	5	20	7	0
		23	2	12	0	0
		24	0	13	5	0
		25	0	12	6	0
		26	3	15	2	0
		27	2	20	1	1
		28	8	10	6	0
		29	5	17	1	0
		30	3	12	6	0
Total			77	383	167	1

Origin of double lysogens as in Table 4 and Table 7, row 3. Each *gal*$^-$ strain recorded was derived from restreaking a separate *gal*$^+$ colony which was itself doubly lysogenic.

TABLE 6

Transduction into K12 gal⁻ ($\lambda m_5 h+ co_2$ imm$^\lambda$) *by lysate from K12* gal⁻(λm_5+h co_2+ imm$^\lambda$) (λgal+ co_2+ imm$^\lambda$) *as a function of multiplicity of infection*

Multiplicity of infection (range)	Transduction clones			
		Lysogenic, carrying *h*		
	Lysogenic not carrying *h*	*gal*+/*gal*⁻ m_5+ *h* co_2+ *imm*$^\lambda$	*gal*+/*gal*⁻ m_5/m_5+ *h*/*h*+ co_2/co_2+ *imm*$^\lambda$	Defective
2 to 6	199	3	7	1
0.4 to 0.9	465	1	6	0
0.004 to 0.009	476*	0	0	0

The first row contains previously published data (CAMPBELL 1957). The one defective was revertible and hence apparently a spontaneous defective not related to transduction. The second and third rows are the combined totals from four experiments.

* 30 clones were analyzed for prophage content. Twenty-six were (m_5h^+ co_2/co_2^+); three were (m_5h^+ co_2); one was (m_5h^+ co_2^+).

for their content of alleles of the *h* gene. The data of Table 6 show that within the precision of these data, the frequency of occurrence of the h allele among the *gal*+ colonies approaches zero as zero multiplicity is approached. The strains previously studied must therefore have originated by multiple rather than by single infection.

This does not prove that they are triple lysogens. For example, the structure λm_5+*hgal*+co_2+*imm*$^\lambda$ might conceivably be formed as a result of recombination at the time of lysogenization in a cell infected simultaneously with λm_5+*h*co_2+ *imm*$^\lambda$ and λ*gal*+co_2+*imm*$^\lambda$. The only rigid demonstration of triple lysogeny is the simultaneous appearance in one cell of three members of a series of multiple alleles. The characters *imm*$^\lambda$, *imm*^{434}, and co_2 come close to qualifying, because the distance between co_2 and the c_I region is quite small.

We therefore infected K12 *gal*⁻ ($\lambda m_5 h+co_2 imm^\lambda$) with λ*gal*+co_2+*imm*$^\lambda$ using λm_5+*h*co_2+*imm*^{434} as helping phage. The results are shown in Table 7. Seven out of 19 strains have the content of prophage alleles which is expected of triple lyso-

TABLE 7

Production of triple lysogens

Number of transduction clones	Content of prophage alleles	Presumed structure
7	m_5+ *h* co_2+ *imm*$^\lambda$/*imm*^{434}	K12 *gal*⁻(λm_5+*h*co_2+*imm*^{434}) (λ*gal*+co_2+*imm*$^\lambda$)
7	m_5/m_5 + *h*/*h*+ co_2/co_2+ *imm*$^\lambda$/*imm*^{434}	K12 *gal*⁻($\lambda m_5 h+co_2 imm^\lambda$) ($\lambda m_5 h co_2$+*imm*^{434}) ($\lambda$*gal*+$co_2$+*imm*$^\lambda$)
1	m_5h+ $co_2 imm^\lambda$	K12 *gal*⁻($\lambda m_5 h+co_2 imm^\lambda$) ($\lambda$*gal*+$co_2$*imm*$^\lambda$)
4	m_5h+ co_2/co_2+*imm*$^\lambda$	K12 *gal*⁻($\lambda m_5 h+co_2 imm^\lambda$) ($\lambda$*gal*+$co_2$+*imm*$^\lambda$)

Recipient: K12 *gal*⁻($\lambda m_5 h+co_2 imm^\lambda$).

Donor: K12 *gal*⁻(λm_5+*h*+co_2+*imm*$^\lambda$) (λ*gal*+co_2+*imm*$^\lambda$). Induced lysate used at multiplicity of 3×10^{-2}.

Helping Phage: λm_5+*h*co_2+*imm*^{434}. Used at a multiplicity of 2.2.

gens. In the rest, either there has been substitution of the original phage by the helping phage or else the helping phage has not lysogenized.

DISCUSSION

The observed data on transduction and segregation are in good agreement with the idea that the transductions are invariably mediated by a specific type of phage characterized by the absence of certain genes and the presence of others derived from the host. That this particle results from a genuine hybridization process between viral and host genomes rather than a casual association between fragments derived from the two is indicated by (1) the fact that the same specific portion of the phage genome is always missing and (2) the correlated loss of the *gal*$^+$ character and the allele of the c_I region which entered a syngenote in association with it.

Our knowledge of the simplest cases of transduction and segregation, while not yet complete, is now in a reasonably satisfactory state. Under conditions of single infection, sensitive recipients regularly yielded defective heterogenotes and lysogenic recipients yield lysogenic heterogenotes. The former regularly segregate sensitive *gal*$^-$ substrains. Generally, the *gal*$^-$ derivatives from the latter are singly lysogenic and contain the alleles of the *c* region from the prophage originally carried.

There are many more complex cases the analysis of which is still inadequate. The strains we originally chose for detailed study have now proved to be triple lysogens, which show a frequent joint loss of one defective and one active prophage. Preliminary examination of the segregants from the triple lysogens from Table 7 indicates that this is not the only possible pattern but that other types of joint loss can also occur. These effects presumably reflect the physical relationship between the prophages, but we cannot yet form a clear picture of precisely what is happening.

The immunity genes promise to be very useful tools for further work because of the ease of scoring in defective as well as true lysogens. We are currently investigating the properties of double defective lysogens of the presumed structure K12 $gal_1^-gal_2^-$ (λgal_1^+ $gal_2^-imm^{\lambda}t$) ($\lambda gal_1^-gal_2^+imm^{434}t^+$). Such studies should help in elucidating the genetic map of the transducing prophage.

SUMMARY

1. The comparative properties of the bacteriophages λ and λ-434 hybrid with respect to transduction of the galactose markers have been studied. Low and high frequency transductions with the hybrid are similar to those with λ.

2. Heterogenotes produced from lysogenic recipients usually carry alleles from the c regions of both the donor phage and the prophage of the recipient, and exhibit a correlated loss of the *gal*$^+$ character and the c region allele derived from the donor phage.

3. In mixed infection with a transducing phage, a nontransducing phage will increase the probability of transduction. This increase does not occur if the

recipient carries a prophage with the same immune specificity as the nontransducing phage.

4. Triple lysogens carrying two active and one defective prophage have been produced by mixed infection of lysogenic recipients.

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THE MUTABILITY OF 18 MEV ELECTRONS APPLIED TO DROSOPHILA SPERMATOZOA[1]

I. H. HERSKOWITZ,[2] H. J. MULLER, AND JOHN S. LAUGHLIN

Department of Zoology,[3] Indiana University, Bloomington, Indiana, and Department of Physics, Memorial Center for Cancer and Allied Diseases, New York, New York

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IN work on the mutagenic effectiveness of different ionizing radiations (see HOLLAENDER 1954 for summaries of advances in this field) it is desirable to use radiations of maximal homogeneity and measureability and also to test organisms providing groups of cells for study that are genetically and physiologically as uniform and constant as possible, and that allow the study of diverse types of genetic changes in the same batch of material. In the present study on translocations, sex chromosome losses, and sex-linked recessive lethals induced by 18 mev electrons administered to mature sperm of *Drosophila melanogaster* the attempt was made to meet these requirements. It had, moreover, become clear as a result of recent work on the differential mutagenic susceptibility of sperm irradiated at different times prior to ejaculation (LÜNING 1952; MULLER, HERSKOWITZ, ABRAHAMSON and OSTER 1954; ABRAHAMSON and TELFER 1954) that a redetermination of the frequency-dosage relationship between penetrating ionizing radiation and these different types of change was necessary. Some of the results of the present study have already been recorded in abstract form (HERSKOWITZ, MULLER and LAUGHLIN 1956).

MATERIALS AND METHODS

The standard "multipurpose" technique (MULLER 1954) was employed to detect the three different types of mutation. The P_1 females (X·Y *InEN y; st*) were collected as virgins over a period of about a week, stored in vials containing normal (protein-rich) culture medium, and then mated at random to P_1 males (sc^8·Y/*y In49 B; bw*^{D}) also collected over a similar period of time. After a mating period of two days the males were discarded and the females (almost all of which had been inseminated) placed in vials containing protein-deficient medium (sugar-agar-water) for a few days. The females were then divided at random into groups, one not to be irradiated and the others to be treated with different doses of the fast electrons.

The electron irradiations, carried out at the Memorial Center at room temperature, were given to motile, well-aerated females enclosed in 1 cm polystyrene at the rate of about 50 rads/min with an energy of about 18 mev. The flies were

[1] This work was supported by a grant from the Atomic Energy Commission (Contract AT (11-1)-195).

[2] Present address: Department of Biology, Saint Louis University, St. Louis, Missouri.

[3] Contribution No. 672.

given total doses of 600, 1500 or 3750 rads. The groups receiving lower doses were exposed, whenever feasible, during part of the time those receiving higher dosages were treated. Thus there were interruptions of about two minutes duration in the longer treatments to remove the groups receiving the shorter treatments. The error in electron dosimetry is estimated to be less than two percent.

After the irradiation the females in all four groups were placed into vials containing normal culture medium in such numbers as would result in approximately the same degree of crowding of larvae, namely 1, 1, 2, and 30 females for the control, 600, 1500, and 3750 rads groups respectively. The attainment of this optimal degree of crowding further required these females to be shaken into fresh vials every 2, 2, 4, and 4 days, respectively.

Complete loss of either paternal sex chromosome or partial losses involving the removal of y^+ were recognized through the appearance of exceptional F_1 males having yellow body and non-Bar eyes. Sex-linked recessive lethals were detected as follows. The F_1 females (of heterozygous Bar phenotype) were bred in separate vials with F_1 or P_1-type males; all cultures in which the next generation (F_2) included no Bar males were tested further as a check for possible lethals. The presence of a lethal was considered proved only after virgins like the F_1 female (obtained in F_2 or later generations) were found (after mating with X·Y *InEN* $y/$ sc^8·Y males) to have produced no Bar-eyed sons in cultures that gave a total of more than 100 Bar-eyed daughters. Translocations between chromosomes II and III were detected in F_2, from matings of individual F_1 males to virgins like the P_1 females, by the failure of any progeny (numbering at least 14) to show II-III recombination. Doubtful cases of translocation were tested by repetition of the same type of cross for another generation.

The breeding was carried out at 25±1°C.

RESULTS AND DISCUSSION

The frequencies of losses of all or a part of the paternal sex chromosome following different treatments, resulting in y males, are given in Table 1. Because of

TABLE 1

Sex chromosome losses following different treatments with electrons

Treatment in rads	No. losses		Percent induced on basis of		On basis of results of 3750 rads and A	
	Total no. males	Percent	*S*	*A*	Percent expected on linearity	Percent expected on 3/2 power rule
Unirradiated controls						
Simultaneous (*S*)	6/1,608	0.37±0.15	.....	.....	.....	.....
Accumulated (*A*)	38/21,929	0.17±0.03	.....	.....	.....	.....
Treated						
600	11/3,295	0.33±0.10	−0.04±0.17	0.16±0.11	0.49±0.04	0.20±0.02
1,500	90/8,880	1.01±0.11	0.64±0.19	0.84±0.12	1.22±0.05	0.77±0.06
3,750	170/5,252	3.23±0.24	2.86±0.29	3.06±0.24	3.06±0.24	3.05±0.24

the perhaps fortuitously high rate of losses in the concurrent unirradiated control and the large statistical error due to small sample size, the frequencies induced by the irradiations were estimated in two ways: (1) by subtracting the simultaneously run control value (S) and (2) by subtracting the average (A) obtained by combining these control results with those of the unirradiated controls of earlier experiments in which the same stocks were used; this average giving a frequency only half as high as S. When S was used to determine the induced rates we found the anomalous condition of a negative value for the lowest dose (a result giving further evidence that the S value represented a deviation in the high direction); and the frequencies of losses at all doses used were found to differ from one another by amounts significantly greater ($P < .01$) than those expected on the basis of a linear relationship between mutation rate and dose. When A was used, the probability for rate-dose linearity in comparisons of results obtained with 600 rads and 1500 rads was 0.11, while for comparisons between 600 rads and 3750 rads and also between 1500 rads and 3750 rads, the probability value was $< .01$.

Thus it can be concluded that the over-all frequency of sex chromosome losses of the diverse types here detected rises with electron dose at a rate significantly above linearity. Moreover, the frequency seems to vary approximately as the 3/2 power of the dose, like that of known multibreak rearrangements (translocations, gross deletions, gross inversions) but unlike what had been found in the earlier investigations of MULLER (1940) and PONTECORVO (1940, 1941) and in some later studies on sex chromosome losses when the spermatozoa were irradiated while in the male. However, in later (unpublished) studies of our group in which the genetic scheme followed in this report was used and irradiation was carried out on the inseminated females, a frequency-dosage relation similar to the one reported here was obtained. An interpretation of the seeming discrepancy may be found in the composite nature of this group of changes in which large deletions of the paternal y-containing X chromosome form a high proportion of the changes under the genetic setup used. A second possibility is a difference in susceptibilities of different germ cell stages to the production of the diverse types of change. There may also have been a heterogeneity of the material and selective effects. The topic is to be discussed in more detail in another paper. We believe that our material in the present study was especially uniform as to stage, susceptibility, and selective effects, and that most of the losses found represented multihit phenomena.

The frequencies of II–III translocations following different irradiation treatments are presented in Table 2. Statistical tests of whether the different mutation rates vary linearly with dose gave values of $P = .04$ for the comparison of 600 rads with 1500 rads, and $P < .01$ when 3750 rads was compared with 1500 rads and also when 3750 rads was compared with 600 rads, the differences being greater than expected on linearity. In fact, the frequency of these translocations, like that of the sex chromosome losses, varies approximately as the 3/2 power of the dose, and the agreement is in this case more striking than for the losses since larger numbers of "events" are involved. According to the 3/2 power relation

TABLE 2

Reciprocal II–III translocations following different treatments with electrons

Treatment in rads	No. translocations / No. tests	Percent induced
600	36/3114	1.16±0.19
1500	107/2620	4.08±0.39
3750	203/1210	16.8 ±1.1

TABLE 3

Sex-linked recessive lethal mutations following different treatments with electrons

Treatment in rads	No. males tested	No. F_1 cultures yielding different no. lethals						No. lethals / No. tests (Adjusted*)	Percent found* (Adjusted percent induced)
		1	2	3	4	5	6		
Unirradiated Controls	21	0	0	0	0	1	0	5/ 484	1.0
								(0/ 472)	(0.0)
600	65	15	11	1	0	0	2	52/1587	3.3
								(40/1408)	(2.8±0.4)
1500	332	42	19	2	1	1	0	95/2076	4.6
								(90/2051)	(4.4±0.5)
3750	1500†	..	..	..	..	..	..	89/ 769	11.6
								(85/ 765)	(11.1±1.2)

* Numbers and percents in parenthesis omit cultures containing clusters of lethals except in the case of 3750 rads group, in which clusters could not have been found. For the latter group the amount subtracted to obtain the "adjusted" value is the percent found in the form of clusters in the total of all the other cultures (control, 600 rads and 1500 rads).
† Approximately.

the 6.25-fold difference between the highest and lowest doses should produce a 15.6-fold difference in mutation rate, a 14.5-fold difference being observed, while the 2.5-fold difference between the low and intermediate doses and between the intermediate and high doses should give 3.9-fold differences, the differences actually observed being, respectively, 3.5-fold and 4.1-fold. In all these cases, moreover, there is no statistically significant discrepancy between the observed difference and that expected on the 3/2 power rule.

Table 3 gives the results of the tests for sex-linked recessive lethals. Among the controls five lethals were produced among 12 tested F_1 females from a single P_1 female, no other lethals being obtained. Since these lethals were almost certainly derived from sperm of a single male that was mosaic for a lethal or carried a subvitality mutant, it would be more appropriate to exclude the data from this P_1 female, since previous work (MULLER 1946, and unpublished) has shown that the frequency of lethals arising in this way is relatively low. The resultant zero value for the control is shown in parentheses while the "crude" value, without this modification, is shown on the line above it. The 52 lethals obtained from 600 rads treatment also show a similar non random distribution, in that two

vials produced F_1 each yielding six lethals among a total of 179 tested F_1 females from these P_1. As before, we are therefore excluding these lethals in our second line, that shown in parentheses, for this dose, but including it in our first line. The distribution of the 95 lethals in the 1500 rads treatment again indicates that the culture yielding five lethals in 25 tests was due to a pre-existing mutation of this type and we have shown these data according to the same system.

In each of the above cases we have in the first line included all the data because a separation of "clustered" from independently arisen lethals cannot be made for the highest dose (3750 rads) in view of the fact that there were 30 P_1 females per vial, each of which could have produced only a few offspring. According to the results obtained with other treatments, there could have been among the large number of parents required for the 3750 rads treatment about four lethals detected which originated as clusters (all these of course being spontaneous since the treatment was applied too late to give rise to clusters).

The results for induced lethals obtained by the method referred to do not depart significantly from a linear relation. Moreover, they fit a linear relation even better if instead of using the obtained control value of 0.0 percent lethals, the more usual one of 0.2 percent (which in fact is within the range of the error to which the obtained data are subject) were used as a subtrahend in determining "more probable induced values."

The shapes of the curves for all three types of mutations induced by different doses of electrons (Figure 1) are in good agreement with those obtained previously with the multipurpose technique after X-ray treatments of inseminated females (ABRAHAMSON and TELFER 1954; MULLER, HERSKOWITZ and OSTER, unpublished). The question of the "relative biological effectiveness" of the electrons used, as compared with ordinary X-rays, is still left open, since work of a corresponding type carried out with X-rays has thus far been subject to errors of dosimetry that have prevented exact comparisons except for comparisons of the shapes of the curves. The comparisons suggest, however, that the high-energy electrons are somewhat (although not very much) less efficient than the X-rays.

The further question may be asked: do the X-rays and 18 mev electrons have the same relative effectiveness in the production of all three of the classes of changes here considered: losses, translocations and recessive lethals? This question is not answered by the fact that the frequency-dosage curves obtained with the X-rays and the high-energy electrons are alike in shape for losses, for translocations, and for lethals, respectively. Instead one needs to make a comparison of the positions of the points on the curves at any one dose. We have made such comparisons with the data available to us and find that these data do not depart significantly from the values expected on the assumption that both types of radiation are alike in their mutagenic patterns. That is, the data are consistent with the assumption that, for a dose of radiation, producing a given percent of lethals, there is a given (other) percent of losses, and another given percent of translocations, no matter whether X-rays or 18 mev electrons have been used.

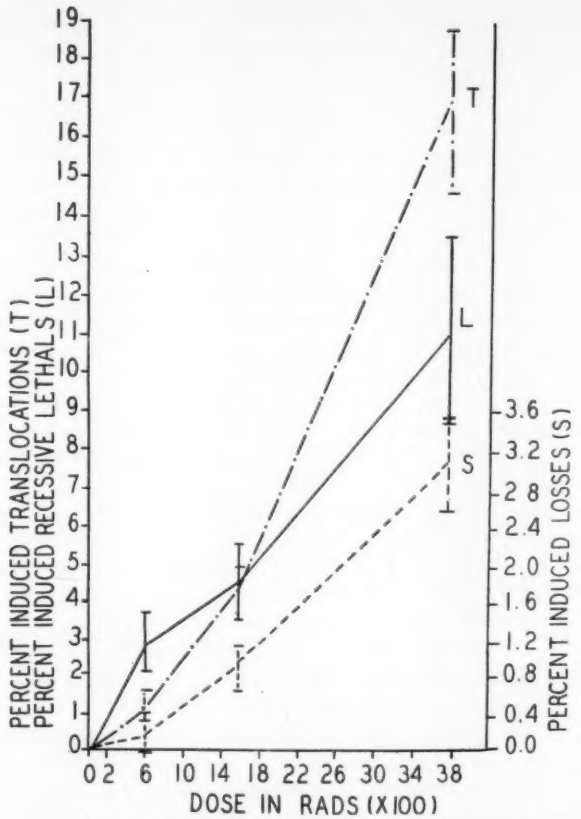
PERCENT INDUCED TRANSLOCATIONS (T)
PERCENT INDUCED RECESSIVE LETHALS (L)
PERCENT INDUCED LOSSES (S)
DOSE IN RADS (X100)
T
L
S
0 2 6 10 14 18 22 26 30 34 38
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
0.0 0.4 0.8 1.2 1.6 2.0 2.4 2.8 3.2 3.6

FIGURE 1.—Percentages of mutations, $\pm 2 \times$ S.E., induced by different treatments of 18 mev electrons. The sex chromosome loss frequencies, connected by broken lines, are corrected for the rate obtained in accumulated controls; the reciprocal II–III translocation frequencies are connected by dot-dash lines; the sex-linked recessive lethal frequencies, joined by solid lines, are adjusted for the control rate in the manner described in the text and Table 3.

SUMMARY

Sperm in inseminated females were either untreated or treated with 600, 1500 or 3750 rads of 18 mev electrons and different types of mutation involving the paternal chromosomes were detected among the offspring. Partial or complete sex chromosome loss increased slightly, but significantly, faster than linearly with dose; II–III reciprocal translocations increased approximately as the 3/2 power of the dose; and sex-linked recessive lethal mutations increased approximately linearly with dose. The shapes of all three curves are in good agreement with previous X-ray results.

The present data do not disagree with (although they do not yet prove) the assumption that the relative biological effectiveness of 18 mev electrons as compared with X-rays is the same for all three classes of genetic changes studied.

ACKNOWLEDGMENT

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INDUCED CHANGES IN FEMALE GERM CELLS OF DROSOPHILA. VI. THE EFFECT OF DEHYDRATION UPON THE X-RAY-INDUCED FREQUENCIES OF CROSSOVER-LIKE EXCHANGES AND OF GROSS CHROMOSOMAL REARRANGEMENTS[1]

IRWIN H. HERSKOWITZ

Department of Biology, Saint Louis University, St. Louis, Missouri

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DEHYDRATION of *Drosophila melanogaster* virgin females before irradiation increases the frequency of mortality for eggs laid approximately the first eight days after mating (HERSKOWITZ 1957). No decision was reached from this work as to whether or not the dehydration-increased egg mortality had a genetic basis. The present experiments aimed to test whether X-ray-induced frequencies of gross chromosomal rearrangements and of crossover-like exchanges are modified by maternal dehydration prior to irradiation. The findings are discussed in relation to those from the earlier study on dehydration-dependent egg mortality.

Part of these results have been summarized elsewhere (HERSKOWITZ 1956).

MATERIAL AND METHODS

Drosophila melanogaster was used. To investigate crossover-like exchanges, virgin females were collected, aged 4–6 days on standard culture medium enriched with brewer's yeast, and divided into two groups at random. One group was left on this culture medium, the other was placed in empty vials to dehydrate the females for 12 hours in a room where the relative humidity was 40 percent. After this time, when about ten percent of the dehydrated group had died, both the normally hydrated (W) and dehydrated (D) females were divided into two subgroups, of which one was unirradiated (C) and the other irradiated (I) with about 2940r of X-rays given at the rate of 1404r/min over a period of 2 min 5 sec.

Irradiation was administered to females inclosed in gelatin capsules, each of which contained 80 females. Treatment was given at 20 ma and at a peak of 200 kv with 1 mm Al filtration. Two hours after irradiation the females (WC, WI, DC, DI) were etherized and placed together with an excess of males into large containers having tops and bottoms covered by dacron netting (see TELFER 1954). Since different containers held different numbers of females the bottom area to be used for egg-laying was made proportional to the number of females introduced by covering an appropriate part of the netting with metal foil. The

[1] The experimental work was performed at Indiana University where it was supported by a grant to DR. H. J. MULLER and associates from the Atomic Energy Commission (Contract AT (11-1)-195).

bottom side was placed on top of sugar-agar food and the surface of the food was seeded with live yeast in Petri dishes. Such egg-laying units and Petri dishes were sealed in paper sacks, except during egg counting and transfers. Fresh Petri dishes were provided twice per 24 hours, usually early in the morning and late in the afternoon, for 20 days. The eggs laid in the Petri dishes through the meshes of the netting were counted soon after the dishes were replaced. At least 36 hours elapsed from the time the dishes were replaced before the medium in the dishes (carrying the larvae) was removed and placed into bottles containing a standard cornmeal, molasses, brewer's yeast, carragar nutrient medium. A thick suspension of live yeast was added to the bottles when the medium in the Petri dishes was placed in the bottle. Subsequently yeast was added again. The same amount of extra yeast was always added to all bottles for all treatments containing individuals from eggs laid in the same time period.

Whenever the number of eggs on a Petri dish exceeded 1000, the medium was divided into the smallest number of approximately equal parts each of which would contain less than 1000 eggs. Each piece was placed in a separate bottle and handled as described. The parents were etherized periodically and the number of living females determined. The number of eggs laid in all 40 half-day periods were counted. However, to limit the labor involved, the eggs for all periods except the 16 following were grown to adulthood: 10, 12, 13, 15, 18, 20, 21, 23, 24, 27, 28, 29, 30, 32, 33, 34; in 39 WC eggs were discarded by error.

The female parents were y·Dp sc^{v1} y^+/y^z *v f car* and the males Y^+/y^z *v f car*. F_1 flies emerging from the bottles within 19 days from the start of egg-laying were classified and scored for sex, body color (carrying y^+ or not), eye color (carrying car^+ or not), and bristle shape (carrying f^+ or not).

The temperature was 25 ± 1°C throughout.

To study gross chromosomal rearrangements, virgin females were obtained, carrying the "snoc" attached-X chromosome (*sc* ct^n *oc ptg car*·sn^{zz} *In49 y*) and a modified Y (y^+ sc^{v1}·Y^s), and kept on brewer's yeast-enriched nutrition for 1½ days. They were then divided into two groups at random; one was left on culture medium and the other was placed in empty vials and allowed to dehydrate for 13 hours. After this time the W and D groups were subdivided to provide unirradiated (C) and irradiated (I) subgroups. WI and DI females were irradiated simultaneously, while motile and well-aerated, with about 2000r delivered at about 1500r/min at a peak of 200 kv and at 20 ma through 1 mm Al and ½ inch thick lucite. At least one hour elapsed after irradiation before the females were etherized and placed with an excess of males (y sc^{s1} *B In49 v*/Y^+) into egg-laying cylinders of the type mentioned. The procedure from this point on was the same as that used in the crossover-like exchange experiment, except that eggs laid only the first eight half-days were collected and the F_1 was scored for appropriate phenotypes. Intra-X gross deletions and, more frequently, half-translocations following detachment of the attached-X's produce non-Bar males and heterozygous-Bar females in the F_1 (the relatively few triplo-X females that

occurred were not included among the cases of gross chromosomal rearrangement).

RESULTS

Table 1 (and Figure 1) gives the percentage of exchanges for the carnation-centromere region among eggs laid in different periods of egg-laying by females dehydrated and irradiated (DI), dehydrated but unirradiated (DC), normally hydrated and irradiated (WI), and normally hydrated but unirradiated (WC). The results for certain half-day oviposition periods are combined in the table in order to provide suitably large sample sizes. The data for the 11th period, however, are given separately since their inclusion with either the "1–9" group (which would give a P value of .075) or the "14 + 16" group (which would still give $P<.01$) may be misleading. For, at the time of irradiation, eggs laid during "1–9" were past the stage of spontaneous crossing over while those from "14 + 16" were not. Since eggs from period 11 could be a mixture of both kinds, their inclusion in one group could hinder detection of the X-ray effect on the groups separately, particularly if the X-ray effect in successive eggs laid was decreasing in the 1–9 period and increasing in the 14–19 period. Table 2 (and Figure 2)

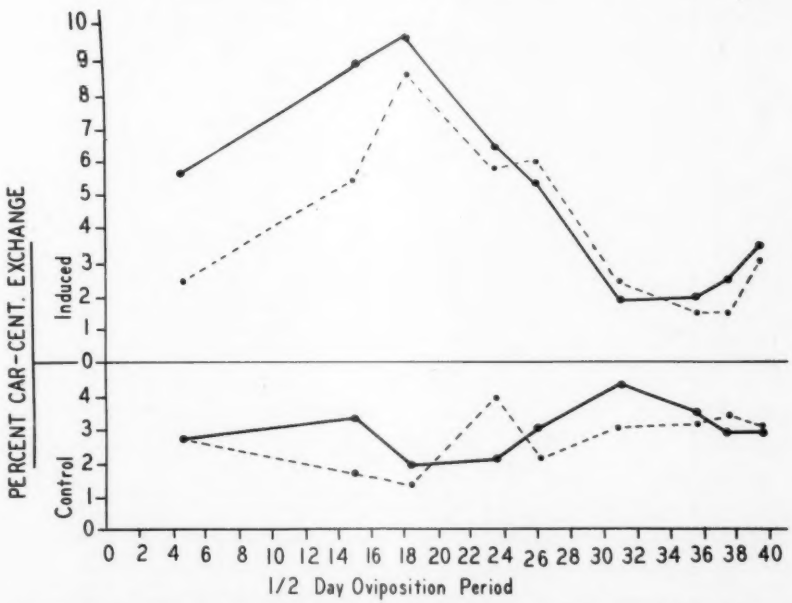

FIGURE 1.—Percent exchange for carnation-centromere region among eggs laid in different periods by dehydrated (solid line) and normally hydrated (broken line) females. Lower two curves are from unirradiated females, upper two curves show the X-ray induced rates. Data from Table 1.

TABLE 1

Percent exchange for car-centromere region among eggs laid in different periods

	1/2-day oviposition periods									
	1–9	11	14+16	17+19	22+25	26	31	35+36	37+38	39+40
DI										
No. F_1	400	247	1846	3765	2874	2633	2863	3766	3252	2974
Percent	8.25±1.34	6.1	12.1±0.76	11.4	8.5	8.3	6.1	5.3	5.3	6.4±0.45
DC										
No. F_1	1967	160	631	756	953	493	552	1021	768	835
Percent	2.69±0.36	2.5	3.3±0.51	1.9	2.1	3.0	4.3	3.5	2.9	2.9±0.58
Percent DI–Percent DC	5.56±1.42	..	8.8±1.04	9.5	6.4	5.3	1.8	1.8	2.4	3.5±0.73
WI										
No. F_1	2531	369	2532	4246	4621	2902	4430	4683	5035	4629
Percent	5.13±0.43	5.7	7.0±0.51	9.8	9.6	8.1	5.3	4.5	4.7	6.0±0.35
WC										
No. F_1	2116	56	668	1072	853	798	974	579	669	569
Percent	2.69±0.35	0.0	1.6±0.49	1.2	3.9	2.1	3.0	3.1	3.3	3.0±0.72
Percent WI–Percent WC	2.44±0.56	..	5.4±0.69	8.6	5.7	6.0	2.3	1.4	1.4	3.0±0.80
Percent induced D–Percent induced W	3.12±1.53	..	3.4±1.25	0.9	0.7	−0.7	−0.5	0.4	1.0	0.5±1.1
P=	<.05	..	<.01	..	..	..	..	..	..	>.05

See text for details.
D=dehydrated, W=normally hydrated, I=irradiated, C=unirradiated.

gives the data for the forked-carnation region in the different egg-laying periods. Table 3 (and Figure 3) presents the data for both these regions in terms of successive groups of eggs laid. The numbers of living females at the start of the experiment, the 22nd half-day, the 37th half-day, and the 40th half-day were for

TABLE 2

Percent exchange for f-car *region among eggs laid in different periods*

	1/2-day oviposition period									
	1–9	11	14+16	17+19	22+25	26	31	35+36	37+38	39+40
DI	3.8±0.96	3.7	4.9	5.5	5.3	5.8	4.0	3.6	3.2	2.9
DC	4.7±0.48	1.9	3.5	3.2	3.3	2.6	3.4	3.6	3.1	3.8
DI–DC	−0.9±1.09	..	1.4	2.3	2.0	3.2	0.6	0	0.1	−0.9
WI	4.2	2.4	4.5	5.8	6.0	4.9	3.3	3.3	2.7	2.8
WC	4.4	5.4	2.7	2.4	2.3	2.9	3.2	3.3	2.7	3.9
WI–WC	−0.2	..	1.8	3.4	3.7	2.0	0.1	0	0	−1.1
Induced D–induced W	−0.7	..	−0.4	−1.1	−1.7	1.2	0.5	0	0.1	−0.2

Symbols same as in Table 1.

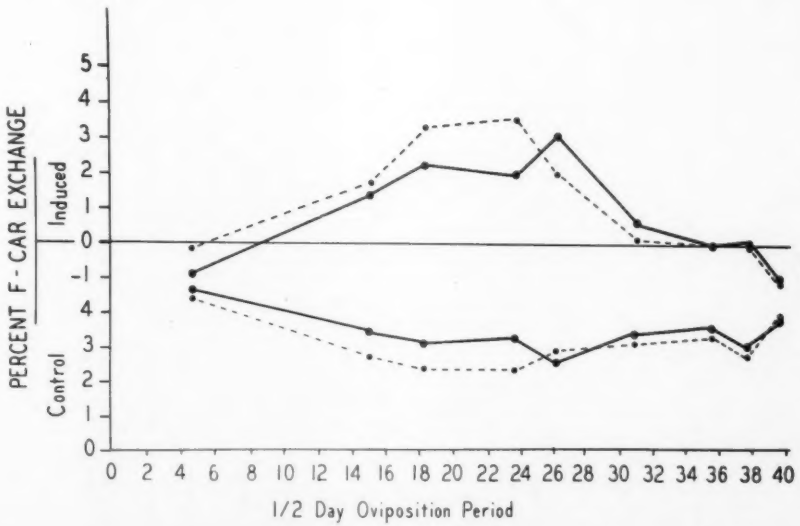

FIGURE 2.—Percent exchange for forked-carnation region among eggs laid in different periods by dehydrated (solid line) and normally hydrated (broken line) females. Lower two curves are from unirradiated females, upper two curves show the X-ray induced rate. Data from Table 2.

TABLE 3

Percent exchange in successive groups of eggs laid

1/2-day oviposition periods	1–9	11	14+16	17+19	22+25	26	31	35+36	37+38	39+40
DI Range of eggs	1–47	48–55	68–98	95–143	162–230	251–272	362–382	446–479	481–514	505–546
DC										
E*	1–8	8+9	9+11+14	14+16+17+19	19+22+25	25+26	31	35+36	36–38	36–40
No. F_1	1516	690	914	1387	1438	1065	552	1021	1026	1861
Percent car-cent.	2.9	2.2	2.7	2.5	2.1	2.4	4.3	3.5	3.0	2.9
Percent f-car	4.6	4.9	4.6	3.3	3.2	2.9	3.4	3.6	3.3	3.5
Induced percent†										
car-cent.	5.4	3.9	9.4	8.9	6.4	5.9	1.8	1.8	2.3	3.5
f-car	—0.8	—1.2	0.3	2.2	2.1	2.9	0.6	0	—0.1	—0.6
WI Range of eggs	1–68	67–77	90–128	115–172	181–274	277–303	403–429	482–519	520–559	548–598
WC										
E*	1–7	8+9	9+11+14+16	14+16	17+19+22	22+25+26	31	31	31	35+36
No. F_1	1760	356	910	668	1478	1651	974	974	974	579
Percent car-cent.	2.6	3.4	2.3	1.6	1.8	3.0	3.0	3.0	3.0	3.1
Percent f-car	4.5	3.7	3.2	2.7	2.5	2.5	3.2	3.2	3.2	3.3
Induced percent†										
car-cent.	2.5	2.3	4.7	8.2	7.8	5.1	2.3	1.5	1.7	2.9
f-car	—0.3	—1.3	1.3	3.1	3.5	2.4	0.1	0.1	—0.5	—0.6

Symbols same as in Table 1.
* Periods whose data were combined to obtain control values for comparable eggs in irradiated.
† Obtained by subtracting from the DI or WI values in Tables 1 and 2 the DC or WC values in this table.

DI, 208, 174, 159, 151; for DC, 51, 36, 33, 33; for WI, 300, 251, 214, 209; and for WC, 37, 36, 22, 21.

Table 4 gives the number of eggs laid by attached-X females after different treatments. Table 5 shows the gross chromosomal rearrangement rates obtained from these eggs. The initial numbers of parent females and the numbers alive at the end of the eighth half-day were, respectively: DI 283, 275; DC 27, 27; WI 80, 64 (most of the difference of 16 flies did not die but were released accidentally); and WC 10, 9.

TABLE 4

Number of eggs laid by attached-X females after different treatments

	Egg-laying half-day periods					
	1–3	4–5	6–7	8	Total	Range of eggs/female
DI	9,030	5,520	10,472	9,165	34,187	121–124
DC	929	984	1,256	462	3,631	135
WI	2,267	1,278	1,937	2,161	7,643	96–119
WC	529	413	408	249	1,599	160–178

Symbols same as in Table 1.

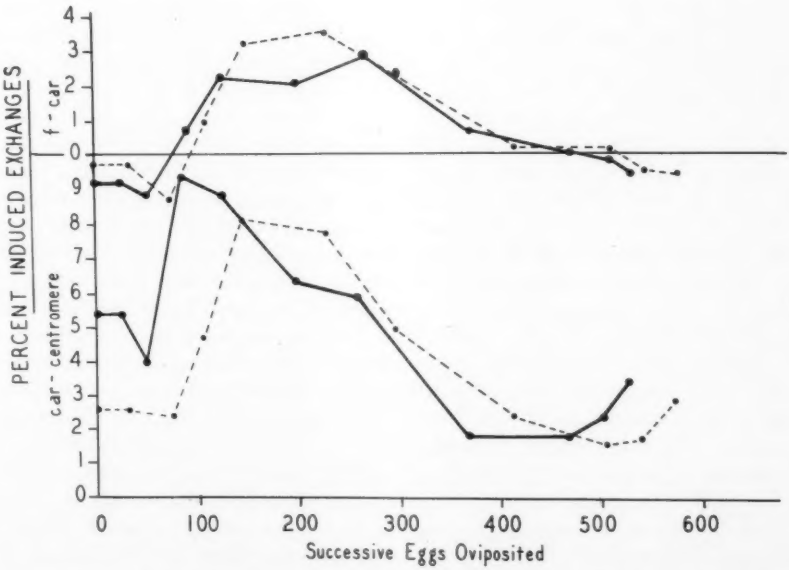

FIGURE 3.—Percent exchange induced by X-rays in successive eggs laid by dehydrated (solid line) and normally hydrated (broken line) females. Lower two curves are for the *car*-centromere region, upper two curves are for the *f-car* region. Data from Table 3.

TABLE 5

Incidence of gross chromosomal rearrangements (T) in F_1 following irradiation of attached-X females. No T were found in the controls

	Half-day egg-laying periods				
	1–3	4–5	6–7	8	Total
DC					
No. ♀♀	189	232	206	82	709
No. ♂♂	161	189	192	91	633
WC					
No. ♀♀	115	73	73	34	295
No. ♂♂	90	76	68	33	267
DI					
No. T ♀♀	22	25	29	16	92
Total No. ♀♀	669	733	1,166	821	3,389
Percent T ♀♀	3.3	3.4	2.4	2.0	2.72
No. T ♂♂	13	7	12	6	38
Total No. ♂♂	650	807	1,139	760	3,356
Percent T ♂♂	2.0	0.9	1.1	0.8	1.13
WI					
No. T ♀♀	3	5	3	0	11
Total No. ♀♀	121	171	239	85	616
Percent T ♀♀	1.4	2.9	1.3	0	1.79
No. T ♂♂	1	0	1	0	2
Total No. ♂♂	105	148	248	76	577
Percent T ♂♂	1.0	0	0.4	0	0.35

Symbols same as in Table 1.

DISCUSSION

Because dehydration or X-ray dosages larger than 2300r can delay egg-laying, it is necessary to compare the results from different treatments with reference both to the time of egg deposition and to the sequence in which eggs were laid (HERSKOWITZ 1957). It should be noted that during dehydration no nutrient was provided. However, because females were given abundant nutrient before dehydration and the period without food was so brief, and in view of the fact that females given only water for 13 hours lay as many eggs as those kept continuously on complete nutrient (HERSKOWITZ 1957), it is safe to conclude that the present experiments could involve effects due to lack of water but not of other nutrients. When the raw data obtained in the present work were analyzed, no significant differences were observed between the sexes, or between the reciprocal classes of exchange or nonexchange individuals for a chromosome region. Accordingly, the data in the tables have been combined in these respects.

For the carnation-centromere region the exchange rate was higher in DI than in DC and higher in WI than in WC in every period studied (Table 1, Figure 1) as well as in successive groups of eggs (Table 3, Figure 3). For eggs laid during the first nine half-days and also in periods 14+16 the X-ray-induced exchange frequencies were significantly greater from the dehydrated (D) than the nor-

mally-hydrated (W) females. Thus the increase occurred in eggs past the stage of normal meiotic crossing over as well as in eggs existing during and before that stage. When the data are arranged according to successive batches of eggs they show a tendency of exactly the same kind. However, in eggs laid after the 16th half-day (and starting roughly with the 100th egg laid in both DI and WI) the dehydration effect is absent even though the X-ray effect remains. Thus the dehydration effect on exchanges lasted for approximately eight days, a period during which dehydration was shown to produce a greater X-ray-induced egg mortality (see Table 3 in HERSKOWITZ 1957).

For the forked-carnation region the results are in some respects similar and in others different from those for the carnation-centromere region. They are similar for eggs laid in periods 14+16 and later (after the 100th egg was laid) in that irradiation increased exchanges until about the 30th egg-laying period after which the effect was reduced or absent. They differ in that while the induced X-ray rate in the first 20 or so periods (Figure 2) after dehydration seems greater than after no dehydration, this is not supported when the data are arranged according to successive egg batches (Figure 3). This failure to obtain a significantly greater number of exchanges from DI females may be due to some basic difference between the *f-car* and *car*-centromere regions, or to the circumstance that induced rates for the former region were not high enough above control values to show a clear enhancement by dehydration. That there may be some fundamental difference between the two regions is suggested by what appears to be a response to radiation of the *f-car* region (which is *opposite* from the *car*-centromere region) in eggs laid in periods 1–9 (or as seen in the first 50 or so eggs laid). For the *f-car* region one finds not only the absence of an increase in exchanges, but what appears to be a *decrease* in exchange frequency after irradiation of both W and D females. A possible decrease was found for this region also in the eggs laid the first two days after irradiation with about 2940r in earlier work (HERSKOWITZ and ABRAHAMSON 1957).

Does the present work show any effect of dehydration on the spontaneous crossover rate (as detected from WC and DC females)? It should be noted that eggs laid beginning with the 12–16 half-days after mating would be in the stage of spontaneous crossing over at the time the mothers were or were not dehydrated. Examination of Tables 1–3 does not give any indication that dehydration has *decreased* the rate of spontaneous crossing over, although such an effect was reported by IVES, FENTON, YOST, and LEVINE (1953) for an autosome after pupae were dehydrated.

The experiment with attached-X females (Table 4) showed that the greatest number of eggs was laid by WC females, that DC females laid more than DI, and WC more than WI. In this experiment DI females did not lay fewer eggs than did WI females. Accordingly, provided the rearrangement rate for DI is higher than for WI, it is permissible (see discussion in HERSKOWITZ 1957) to compare the total induced rate of 1.93±0.17 percent (130/6745) for DI with the rate of 1.04±0.29 percent (13/1256) for WI, as well as to compare the results obtained

for corresponding periods of egg-laying. The total frequencies have less than a one percent chance of representing random samples of the same population. Moreover, in all eight brood comparisons that can be made from the data in Table 5, DI had a greater mutation frequency than WI. It is therefore concluded that gross chromosomal rearrangement frequency (composed primarily of half-translocations) is enhanced if X-radiation is preceded by dehydration.

The facts that dehydration increases the crossover-like exchange rate for the first eight days after irradiation and that gross chromosomal mutation rate is also enhanced make it reasonable to propose that the increase in egg mortality obtained when dehydrated females are irradiated has a genetic basis. Since both the crossover-like exchanges and the gross rearrangements induced by X-rays are already known to be multihit events, and since some of the former and all of the latter result from multiple breaks in chromosomes, it is further proposed that dehydration produces its effect on egg mortality by increasing rearrangement frequency. One way by which dehydration might accomplish this would be by shrinking the nucleus, thereby bringing independently produced broken ends closer to each other, proximity of such ends increasing the likelihood of interchange and decreasing the chance of restitution.

SUMMARY

Dehydration of *D. melanogaster* females significantly increases the rate of crossover-like exchanges induced by X-rays in the proximal region of the X chromosome (between carnation and the centromere). This dehydration effect was found in eggs laid both at the beginning and end of the first eight days after irradiation; that is, periods when the eggs at the time of irradiation were past, and before or during, the stage of spontaneous crossing over, respectively.

The frequency of gross chromosomal rearrangements, as detected by detaching attached-X's, induced by X-rays is also increased by prior maternal dehydration.

These results permit the hypothesis that the increase in egg mortality, obtained earlier when dehydration preceded X-raying, has a genetic basis, and that on this view it is to be considered as resulting from dominant lethal mutations.

Although no cytological study was made, it is suggested that the dehydration effect may have been produced by shrinking nuclei, resulting in an increased chance for broken ends to interchange rather than restitute.

ACKNOWLEDGMENT

I wish to thank Miss Colene Ruch and Mrs. Betsy Bardwell for their very capable assistance in performing these experiments.

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FURTHER STUDIES ON CONVERGENT IMPROVEMENT IN CORN[1]

G. F. SPRAGUE, W. A. RUSSELL AND L. H. PENNY[2]

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THE breeding practices and production technics in the utilization of hybrid corn have developed much more rapidly than our complete understanding of the genetic mechanism of the heterosis involved. The procedures in general use would be expected to have a rather high degree of efficiency if dominance were the primary type of gene action involved. On the other hand, if overdominance were the primary type of gene action, some of the procedures used would be of limited effectiveness and others would be essentially worthless. For example, new inbred lines are customarily given a preliminary evaluation in topcross trials (inbred × open-pollinated variety) to measure average combining ability. Information on average combining ability is meaningful if partial or complete dominance is of importance. However, information on average combining ability would have limited predictive value if overdominance were of major importance. Other procedures used may exhibit similar but less extreme variation in efficiency under the two contrasting assumptions regarding the types of gene action involved in heterosis. Thus, from the standpoint of either plant breeding or genetic theory, it would be desirable to have a more complete understanding of the relative importance of the various types of gene action which may be involved in yield heterosis.

The present paper is concerned with studies on convergent improvement. Convergent improvement is one of the procedures that has been suggested as offering promise of providing critical information on the relative importance of various degrees of dominance.

The method assumes that all longtime inbred lines are lacking in important attributes that combine to produce maximum yield. A high yielding single cross is chosen and crossed to one of the parental lines through several successive generations. During the backcrossing selection is practiced for general vigor and for the desirable attributes contributed by the nonrecurrent parent. After n generations of backcrossing the sublines are heterozygous at all loci where selection has been effective in retaining desirable characteristics of the nonrecurrent parent. Self-pollination is then practiced for two or more generations to fix as many as possible of these desirable alleles in a homozygous condition. If the process is done reciprocally, the new lines A(B) and B(A) will differ from the original

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[2] Principal Agronomist, U. S. Dept. Agr., Assoc. Prof., Iowa State College, and Agronomist, U. S. Dept. Agr.

parents A and B by certain gene substitutions. If selection has been effective, the new lines A(B) and B(A) should be superior to the original parents in yielding ability.

The recovered lines A(B) and B(A) may then be used as parental material for a repetition of the entire procedure. If successful gene transfers can again be accomplished, one would expect the eventual convergence of all of the desirable attributes of the two original lines into a single inbred strain.

Critical evidence on the type of gene action involved is provided by comparisons of the lines in single cross combinations after each cycle of reciprocal back crossing or convergent improvement. Obviously a range in yield is to be expected at both the inbred and hybrid level. However, if any of the substrains of the recovered lines A(B) and B(A) are superior to the original lines A and B, and if single crosses between such superior substrains are equal or superior to the original single cross A × B, then complete or partial dominance is indicated. However, if the substrains of A(B) and B(A) which are superior to the original line produce hybrids A(B) × B(A) which are significantly lower in yield than the parental cross, then overdominance may be indicated. Such results could be obtained, however, by certain types of gene substitutions even in the absence of overdominance. The present paper is concerned with evidence obtained from two cycles of the convergent improvement process.

Review of literature

The method of convergent improvement and some of the theoretical considerations were reported by RICHEY (1927). At a later date RICHEY and SPRAGUE (1931) presented data obtained from one cycle of convergent improvement. It was found that the yield of the recovered lines was increased by 13 to 15 percent. The F_1 crosses between recovered lines following three and four generations of backcrossing were equal or superior to the yields of the parental single crosses.

Murphy (1942) reported data indicating superiority of recovered lines after two and three generations of backcrossing. Some of the single crosses between recovered lines yielded significantly more than the original F_1 cross. The data from the studies reported by RICHEY and SPRAGUE and by MURPHY were interpreted as being in agreement with expectations under the dominance hypothesis and in contradiction to expectations under the physiological stimulation or overdominance hypothesis.

The tests conducted by the workers cited above involved only a single cycle of convergent improvement. The results, therefore, are critical for only that portion of the F_1 vigor that was retained in the recovered lines. Proof that dominance is of major importance in the heterosis unaccounted for after a single cycle of convergent improvement may be obtained only by successive successful repetitions of the process.

MATERIAL AND METHODS

The recovered lines resulting from the studies reported by RICHEY and SPRAGUE (1931) were grown at Missouri in 1934 to 1936 as potential parental material for a second cycle of convergent improvement. The material was poorly adapted and two new lines, B2 and K4, were chosen as parental material. The F_1 hybrid was backcrossed reciprocally to the two parental inbreds for three sucessive generations using standard pedigree culture procedures. The various sublines were then self-pollinated for three generations to fix the desirable genes retained by selection during the backcross generations. The recovered lines were then evaluated in single cross combinations. The results obtained from this first cycle of convergent improvement were similar to the results presented earlier by RICHEY and SPRAGUE (1931) and by MURPHY (1942), and the details will not be presented here. The best of the B2 and K4 recovered lines were designated B2a and K4a, respectively.

The lines B2a and K4a were used as parental material for the second cycle of convergent improvement. Reciprocal backcrossing was practiced for three generations, selecting in each generation for attributes contributed by the nonrecurrent parent. Following the backcrossing procedure, each of the sublines was selfed for two generations and then combined into single crosses. The single crosses and the inbred parents were grown in replicated yield trials. The tests were planted at three locations in each of three years. Due to unfavorable conditions at some locations in each of the three years, one or more of the tests were discarded because of drought damage. Four repetitions of the inbred yield trials and five repetitions of the single cross yield trials were available for summarization.

EXPERIMENTAL RESULTS

The yields of the original and the first and second cycle lines derived by the convergent improvement process are presented in Table 1. The first cycle lines were higher in yield than the original lines. The increase for B2a was 22.4 percent and for K4a, 6.3 percent. Among the second cycle B2b lines, yields ranged from less than that for the original B2 to an increase of approximately 21 percent above the first cycle line B2a. In the K4b series all of the sublines were equal or superior to the original line K4. The second cycle lines having the highest mean yield, K4b-5 and K4b-6, exceeded that yield of the original and first cycle lines by 19 and 12 percent, respectively. These results suggest that the visual selection practiced during the backcrossing and selfing procedures was, on the average, reasonably effective.

The yields of single crosses among the second cycle lines (Table 2) ranged from 53.7 to 92.0 bushels per acre. The constant parent test cross means and the parental line means were significantly associated, the r value being 0.52. The highest yielding single crosses all involved the highest yielding of the B2b lines,

TABLE 1

Yield in bushels per acre for two original and first cycle lines and a series of second cycle lines derived by convergent improvement

Pedigree	Yield bu. acre	Pedigree	Yield bu. acre
B2	28.6	K4	36.5
B2a	35.0	K4a	38.8
B2b–1	22.1	K4b–1	37.2
–2	29.7	–2	39.7
–3	30.3	–3	41.6
–4	31.1	–4	42.7
–5	32.4	–5	43.5
–6	34.4	–6	43.5
–7	35.2		
–8	40.0		
–9	42.3		

Difference necessary for significance at five percent level = 5.5 bu.

TABLE 2

Yield in bushels per acre of single crosses between two original lines and first and second cycle lines derived from convergent improvement

	B2	B2a	B2b–1	B2b–2	B2b–3	B2b–4	B2b–5	B2b–6	B2b–7	B2b–8	B2b–9	Av.
K4	94.4	...	...	...	...	...	...	...	...	...	...	...
K4a	...	91.6	...	...	...	...	...	...	...	...	...	...
K4b–1	...	...	63.4	65.1	75.3	65.0	74.2	82.0	61.8	84.7	79.9	72.4
K4b–2	...	...	71.5	68.6	68.7	65.4	72.4	66.7	74.6	83.6	92.0	73.7
K4b–3	...	...	63.8	71.6	58.0	65.3	66.1	74.9	66.1	78.3	76.5	68.9
K4b–4	...	...	68.9	65.2	53.7	57.9	72.6	60.8	66.6	72.8	89.7	67.6
K4b–5	...	...	67.6	59.6	69.4	61.2	73.4	62.4	75.0	83.7	87.1	71.0
K4b–6	...	...	75.2	67.7	68.1	72.5	74.8	76.4	73.2	88.0	87.3	75.9
Av.	...	...	68.8	65.3	65.5	64.6	72.3	70.5	69.6	81.9	85.4	...

Difference necessary for significance at five percent level = 9.2 bu.

B2b-8 and B2b-9. The K4b parental lines involved in the highest yielding single crosses ranged from the second lowest yielding line K4b-2 to the highest yielding lines K4b-4, K4b-5 and K4b-6. However, it will be recalled that there was no significant difference in yield among this group of lines.

Parent-progeny regressions were calculated for each constant parent group. In the K4 series all of the regressions were positive and nearly equal to 1.0. In the B2 series both positive and negative regressions were obtained ranging in magnitude from +1.24 to −1.87. Both of these extremes are suggestive of overdominance. However, the range in yield among the inbred parents was slight and the number so limited as to make all of these estimates of questionable significance. The b2 values obtained for both series were essentially zero, one being positive and the other negative in sign. b2 values of this magnitude have been interpreted

by HULL (1947, 1952) as indicating either negative or partial dominance rather than overdominance.

DISCUSSION

It was stated in an earlier section that convergent improvement provides a method for making desired gene substitutions, thus improving the performance of an inbred line, without serious modification of hybrid performance. In actual fact single gene substitutions rarely if ever occur. Rather the substitutions involve blocks of chromosomes of varying length. In spite of the intensity of selection practiced during inbreeding, each inbred line so far developed contains many alleles having less than maximum efficiency. The net effect of chromosome block substitutions could be either favorable or unfavorable depending upon the relative number of favorable alleles within the substituted segment.

It would appear that the net effect of the total substitutions involved in the line B2b-1 was unfavorable since the line yielded significantly less than the original parent B2 or the first cycle recovered line B2a. In the lines B2b-2 through B2b-6 the net effect of the chromosome segment substitutions was to reduce the yield level of the inbred below that of the recurrent parent, B2a. In only two of these lines, B2b-8 and B2b-9, did the net effect of the substitutions lead to a significant increase in yield at the inbred level. In the K4b series, in no case did the substitutions lead to significant yield increases.

HENDERSON (1949), using a somewhat restricted definition of dominance, has postulated that certain favorable alleles may have an effect on vigor but show an absence of dominance. The transfer to and accumulation of such alleles in the recovered lines would then lead to increased yields at both the inbred and hybrid level because of homozygosity. This argument would appear to have little validity. Even in those cases in which the gene substitution values for $AA \rightarrow Aa$ and $Aa \rightarrow aa$ are exactly equal, any single heterozygous locus or the sum of all such loci would contribute to heterosis. However, the less favored alleles having such attributes would be expected to be in rather low frequency since such alleles would be most amenable to elimination as a result of the selection practiced during the development of open pollinated varieties.

The results presented here appear to lend some support to the dominance hypothesis as an explanation for yield heterosis. Significant increases in yield were obtained at the inbred level for some of the recovered strains. The single crosses among the best of the recovered lines were slightly lower in yield than the original or first cycle hybrids. However, most of the crosses involving the higher yielding lines were not significantly lower in yield than the parental hybrids. It appears reasonable to assume that, had this study been conducted on a more extensive scale, higher yielding lines and single crosses might well have been obtained.

Additional evidence supporting the dominance hypothesis was the significant correlation between the yields of the recovered lines and the average of their test cross progeny. Furthermore, the parent-offspring regressions for the constant

parent groups indicated either dominance of low yield or partial dominance of high yield rather than overdominance.

SUMMARY

Data are reported on inbred and single cross yields of corn at the completion of two cycles of convergent improvement.

The best of the recovered lines exhibited significant increases in yield amounting to 21 percent in the B2 series and 12 percent in the K4 series.

The yields of single crosses among the recovered lines were less than comparable hybrids between the original parental lines. However, the F_1 crosses between certain of the best recovered lines were not significantly lower in yield than single crosses involving the original parents.

The results are interpreted as providing some support for the dominance theory of heterosis. Additional evidence suggesting the importance of dominance was obtained from the parent and test cross progeny relationships.

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ALLELISM, COMPLEMENTATION, AND PSEUDOALLELISM AT THE DUMPY LOCUS IN DROSOPHILA MELANOGASTER

ELOF AXEL CARLSON[1]

Department of Zoology,[2] Indiana University, Bloomington, Indiana

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FOR recessive mutants, *allelism*, operationally and traditionally, involves three simple tests. First, a newly arisen mutant should be localized to that map region of the chromosome which was previously determined for other members of the series. Second, the mutant should interact physiologically with other members of the series by showing a mutant phenotype in the *trans* compound or mutual heterozygote. Third, no recombination should occur between these alleles. *Complementation*, on the other hand, requires separate map localizations on the same chromosome or location in different linkage groups; it requires a wild, nonmutant, phenotype in the *trans* compound; and, finally, it should yield recombinants from such *trans* pairs of mutants.

In the past decade many of these criteria have been questioned for multiple allelic groups. Originally reported as striking exceptional cases, they have become more frequent every year and have been found in higher plants and animals as well as in microorganisms. These multiple allelic series, failing to satisfy one or more of the traditional tests for allelism or complementation, have been called *pseudoalleles* or complex-loci. (See reviews, LEWIS 1951, 1955; PONTECORVO 1952, 1955).

The dumpy nest of multiple alleles has been useful in investigating the problems of allelism, complementation, and pseudoallelism. Three major approaches have been used in this analysis: (1) crossover relations among the alleles selected for this study, (2) X-ray induction of dumpy mutations and their rates, and (3) the role of position effect and other interactions and modifications of these mutants. The significance of the results and their application to these problems are discussed in the appropriate sections of this paper.

The problem of pseudoallelic nomenclature

The members of the dumpy series show three major recessive mutant effects: an obliquity or truncation of the wings (**o**), eruptions or pits on the thorax, with disturbed patterns of bristles and microchaetae, known as vortices (**v**), and a homozygous lethal effect (**l**). Any combination of these effects can be expressed in the *trans* condition when both chromosomes carry members of this series with

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[1] Present address: Biology Department, Queen's University, Kingston, Ontario, Canada.

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these traits in common. Thus *'o/'ov* shows (**o**), *'v/'olv* shows (**v**), *'ol/'lv* is lethal, and *'o/'lv* shows (+). (See Figure 1 and Table 1 for the major phenotypes seen in this series.) Because the names originally used were unrelated to the phenotypes expressed in terms of these three major effects, it was thought advisable to rename these mutants on the above functional basis.

TABLE 1

Phenotypes expressed at 26° C.

Genotypes	$'l^m$	$'ol^g$	$'olv^1$	$'o^g$	$'lv^1$	$'ov^1$	$'v^2$	+	$'o^{g'}v^2$
$'l^m$	l	l	l	+	l	+	+	+	+*
$'ol^g$	l	l	l	o	l	o	+	+	o*
$'olv^1$	l	l	l	o	l	ov	v	+	l
$'o^g$	+	o	o	o	+†	o	+	+	o
$'lv^1$	l	l	l	+†	l	v	v	+	l
$'ov^1$	+	o	ov	o	v	ov	v	+	ov
$'v^2$	+	+	v	+	v	v	v	+	v
+	+	+	+	+	+	+	+	+	+
dp^{cms}	+	o	ov*	+	v†	ov‡	v‡	+	ov
dp^h	o‡	o	ov	o	v†	ov	v	+	ov
$'o^{g'}v^2$	+*	o*	l	o	l	ov	v	+	ov
$'o^{g'}lv^1$	l	l	l	o	l	ov	v	+	l
$'ol^{g'}v^2$	l	l	l	o	l	ov	v	+	ov*

o = oblique wings.
l = lethal.
v = vortex.
Underline indicates greater intensity of expression.
* Semilethal.
† Blistered wings.
‡ Mild expression, overlapping wild type.

A major difficulty in the comprehension of investigations of pseudoalleles is nomenclature. It is possible, in this series, to employ a flexible system based on the suggestions and practices of several workers in this field (BRIDGES and BREHME 1944; LEWIS 1955; GREEN and GREEN 1956; MULLER and CARLSON, unpublished). First, the alleles, if possible, are designated with names characteristic of their phenotypes or function (e.g., dp^o, dp^v, dp^{ov}). Second, when such a series of mutants is discussed at length, the base (here, *dp*) is omitted and the superscript is used without it (e.g., *o*, *v*, *ov*). Third, a symbol, the apostrophe (*'*), is employed to indicate the omission of the base and to designate the mutant as a member of a pseudoallelic series (e.g., *'o*, *'v*, *'ov*). Fourth, the individual members of this genetic region are given additional superscripts to indicate an allelism at what has thus far proved operationally to be the same sublocus (e.g., $'ov^1$, $'ov^n$, $'ov^{51f}$ are members of the *'ov* sublocus). Fifth, if a phenotype of a mutant should differ from that heretofore usual for its sublocus, then this second superscript may designate this (e.g., $'o^{ov}$ would represent an (**ov**) phenotype at

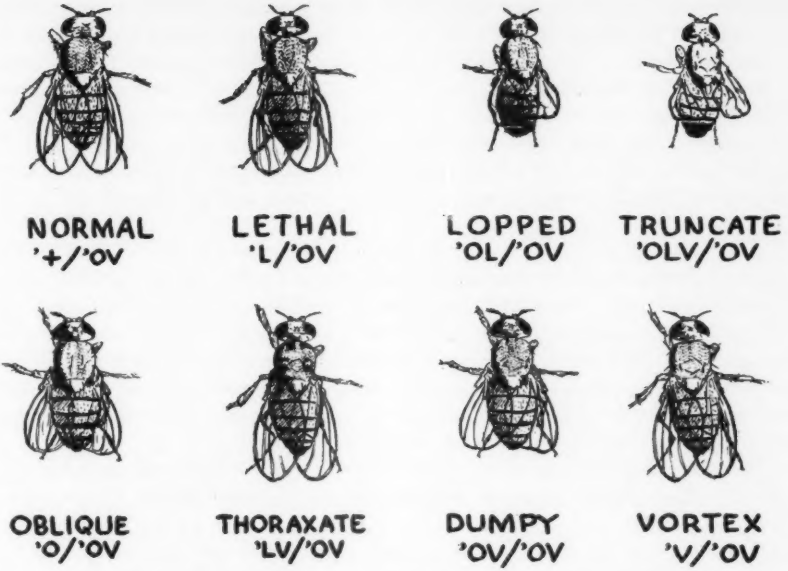

FIGURE 1.—Members of the dumpy series showing the qualitative and quantitative differences in the (o) and (v) expressions possible for them in their heterozygous compounds with *'ov*.

the *'o* sublocus). Sixth, if an already known phenotype is found to be located at a new sublocus, these subloci may be numbered sequentially as found (e.g., *'ov-2* would represent the mutant with an (**ov**) phenotype found at the *'ov-2*, as distinguished from the original *'ov* sublocus, which can be redesignated as *'ov-1*). Although this system has been very satisfactory for the dumpy locus, the problem of nomenclature is an open one in the field of pseudoalleles and this system should be considered a personal one, designed as a mnemonic device to aid the reader in following the various alleles in this study. The numerous members of the dumpy series have been reclassified according to phenotype, using these suggested revisions in nomenclature. (See CARLSON 1958; BRIDGES and BREHME-WARREN, in preparation.)

Historical notes on the dumpy series

The first found mutant of the series later known as dumpy was *truncate* wings (MORGAN 1910), symbolized then as *T* (and later as dp^{T}) but now designated as $'olv^{m}$. This mutant did not breed true and was expressed erratically in spite of many attempts to make it homozygous. An analysis of this mutant (ALTENBURG and MULLER 1920) revealed the following information: first, there was a chief gene in the second chromosome, about locus 13 on the present linkage map, without which the truncate wing phenotype was not expressed. Second, this

chief gene was a recessive lethal. Third, it was in most situations without expression in the heterozygote, but modifiers (intensifiers) in the second, third and X chromosomes of the selected truncate stock were able to bring the chief gene into expression while heterozygous. Fourth, many genetic as well as nongenetic differences, among them those of temperature, modified the expression of truncate wings, i.e., it was a "modifiable character."

During the decade 1910–1920 several other mutants having wing or thorax effects were found: dumpy ($'ov^1$), vortex ($'v^1$), oblique ($'o^1$), lopped ($'ol^1$), and thoraxate ($'lv^1$). At first it was not known that these were members of the same allelic series, but MULLER in 1919 was able to show that the above and truncate, $'olv^m$, could interact with one another in such a way that the compound (*trans* combination) manifested whatever effects the two mutants had in common. Hence these became noted as the first instance of a multiple allelic series affecting qualitatively different characters. For convenience, BRIDGES later (1934) renamed the series, reserving the symbol *T* for translocation instead of truncate, and using the symbol *dp* as a more appropriate designation since these mutants acted for the most part as recessives and showed in *trans* with $'ov^1$.

That these mutants represented variations at a single locus was suggested by the following information: (1) another mutant, humpy (*hy* — II, 93.3) also showed (**o**) and (**v**) effects but did not show interaction with the *dp* series; (2) certain modifiers of the (**v**) effect intensified the (**o**) effect also; and (3) if o^+, l^+, and v^+ represented three adjacent genes then a single event origin (such as a deficiency) would be impossible for these mutants if the three genes were arranged in a linear fashion because, in the sequence *o-l-v*, the mutant *ov* skips the middle member; in the sequence *l-o-v*, *lv* skips the middle member; and in the third possible sequence, *o-v-l*, *ol* skips the middle member. These arguments seemed to support the point mutation nature of each of the various members of this series. The pleiotropism, if this were the case, would be a consequence of a single mutation rather than the combined activities of mutations at a multiplicity of loci. (See MULLER 1939 for references and for discussion of these arguments).

In 1949 and 1952 this question was again raised by FOGEL on the basis of an X-ray study of several second chromosome loci. He found that the dumpy mutants were produced in a much greater frequency than the other selected loci and he interpreted this (in the oral presentation of his 1949 paper) to mean that the dumpy region consisted of a multiplicity of loci. As a means of testing this theory, his students, GOODMAN and LAUFER, attempted to obtain recombinants between $'o^2$ and $'v^2$. GOODMAN (1951, unpublished) was unsuccessful because of the large number of overlaps for the wild type phenotype at the temperature in which the crosses were kept. A similar abundance of overlaps for the wild type was reported by GLASS and SCHMUCKLER (1953). LAUFER (1952, unpublished) reported $'o^2$ to the left of $'v^2$, on the basis of a preliminary recombination test, but was unable to confirm his critical cases by further breeding analysis. Because of these difficulties the initial results were not pursued.

CARLSON (1954) observed an exceptional fly showing strong (**o**) and (**v**)

effects in a culture containing progeny of a female of *'o²/'lv¹ b* composition, which had been mated to an *'ov¹* male. The presence of the outside marker black (*b* — II, 48.5) in the *'lv¹* chromosome of the heterozygous female, and in the recombinant *'o²'lv¹ b* chromosome of this exception suggested that *'o²* was to the left of *'lv¹*. A stock of this double mutant was prepared with a Curly balancer. From it, MULLER extracted the *'lv¹* and confirmed the recombinational nature of this exception. This verified FOGEL's original statement that there was more than one locus in the dumpy series; it also supported LAUFER's suggestion that *'o²* was to the left of *'v²*.

Further recombinants were obtained by CARLSON and by MEYER, with the establishment of *'o²* to the left of *'v²*, of *'ol^m* to the left of *'v²*, and repeating the original finding, of *'o²* to the left of *'lv¹*. Although some 13 recombinants involving pairs with one lethal member (*'ol^m* or *'lv¹*) did not demonstrate separability of the lethal from the locus of the morphological effect that had been originally associated with it, there was one case which could have been interpreted in this way, and one case (presumably of double crossing over or mutation) in which the expected placement of outside markers did not occur. That these results could not conclusively establish or contradict the existence of a third (lethal) locus was in part because the outside markers which were used, Star (*S* — II, 1.3) and Sternopleural (*Sp* — II, 22.0) were too far apart to eliminate the possibility of double crossing over having occurred (with one point of crossing over within the dumpy nest and one between it and one of these markers).

This phase of the work was reported in detail at the 1955 AIBS meetings in East Lansing, Michigan (abstracted in much abbreviated form by MULLER, MEYER, and CARLSON 1955).

MATERIALS AND METHODS

The dumpy alleles used in the research reported in this paper are designated by the functional symbols in Table 3. The outside markers adopted for a critical analysis were echinoid (*ed* — II, 11.0) and clot (*cl* — 16.5). The map distance for the portion of the second chromosome which includes these various mutants is represented diagrammatically:

S	*ed*	*dp*	*cl*	*Sp*
1.3	11.0	13±	16.5	22.0

Originally the flies were raised in shell vials, but for larger counts, half pint cream bottles were used. To maintain the best expression of the mutant phenotypes, the cultures were raised at 26°C. The placing of the outside markers and the choice of the mutant pairs was arranged in such a way as to prevent the possibility of unrecognized stray contamination. In determining crossover rates it was decided to use twice the incidence of wild type recombinants because the occurrence of the double mutants was either reduced from the resulting lowered viability, or in some cases, these double mutants could not be distinguished from one of the members in the *trans* compound used.

TABLE 2

Recombination types

Derivation of *(olv)* type		*(olv)* genotype used for extraction test		Recombinant types extracted
$'o^2/'lv^1$ ♀	⟶	$'o^2'lv^1/+$ ♀	⟶	$'o^2$ and $'lv^1$ only
$'ol^s/'v^2$ ♀	⟶	$'ol^{s'}v^2/+$ ♀	⟶	$'ol^s$ and $'v^2$ only
+ (point mutation)	⟶	$'olv^1/+$ ♀	⟶	no recombination

TABLE 3

Crossover rates involving members of the dumpy series

Alleles used in *trans* female	Double mutant obtained	Reverse mutations or "conversions"	Total verified single crossovers	Total count	Percent crossover frequency
$'o^2/'v^2$	yes	none	23	27,621	0.083
$'ol^n/'v^2$	yes	none	16	22,892	0.07
$'o^2/'lv^1$	yes	none	6	49,692	0.012
$'v^2/'ov^1$	no	none	7*	35,200	0.04
$'lv^1/'ov^1$	yes	1	6*	66,009	0.018
$'ol^m/'ov^1$	no	1	9*	32,124	0.056
$'olv^1/'ov^1$	α	none	4*	26,547	0.03
$'o^2/'ov^1$	no	none	6*	57,207	0.021
$'l/'ov^1$	no	none	2*	2,381	0.167
$'ov^n/'ov^1$	no	1	0	66,313	0
$'ov^{51f}/'ov^1$	no	none	0	51,477	0
$'lv^1/'v^w$	no	none	14*	26,280	0.107
$'olv^1/'v^w$	β	none	6*	7,875	0.152
$'lv^1/dp^h$	no	none	1	3,548	(Inversions present to increase rate of recombination)
$'v^2/dp^h$	no	none	1	2,955	
Total		3	101	485,681	

* Indicates frequency of wild type recombinant should be doubled to calculate crossover frequency.
α Probable; a mutant of *'olv* type occurred in a culture of *'ov* flies. Not yet analyzed.
β Probable; a mutant of *'olv* type occurred in a culture of $'v^w$ flies (MULLER 1957, unpublished). Not yet analyzed.

Crossover analysis of the various members of the dumpy series

At first it seemed reasonable to assume that two regions were involved for the two respective morphological traits: one for the wing effect, another participating in the development of the thorax. This was supported by the preliminary findings that *'o* and *'ol* were to the left of *'v* and *'lv*. They also suggested the possibility of a third locus, *l*, between the *o* and *v*. Since there were three possible (*olv*) genotypes, a series of tests was run to detect the presence of a third locus. Table 2 shows the kinds of recombinations obtained from the (olv) types of different derivation.

From these results (see Table 3 for counts involved) there was no evidence for a separate lethal, *l*, locus. This did not rule out the possible existence of such

lethals lacking the (**o**) and (**v**) effects—indeed, their existence was later discovered by MEYER (1958, unpublished). It did, however, eliminate the possibility of the lethals studied in these cases forming a third lethal region which was approximately midway between the (*o*) and (*v*) regions.

A second difficulty with a two or three sublocus interpretation arose when a comparison was made of the rates of recombination in the various crosses. Recombinants between *'o*^{2} and *'lv*^{1} occurred with only one tenth the frequency of those between *'o*^{2} and *'v*^{2} or between *'ol*^{2} and *'v*^{2}. This meant that one of the lethals, *'lv*^{1}, might possibly be a small rearrangement within the region, or that these pairs of mutants were at more than two subloci. (See CARLSON 1957). An attempt to obtain wild type recombinants arising from crossovers between *'lv*^{1} and *'v*^{2} was successful and showed *'lv*^{1} to be to the left of *'v*^{2}.

Although this meant that a simple *o-l-v* regional sublocus interpretation had to be abandoned, it did not rule out the possibility that a multiplicity of loci might still be arranged in a physiological pattern that corresponded to the phenotypes. For example, if one were to imagine two overlapping gradients, with intensities of effect distributed as shown in the phenotype model of Figure 2, and if the various members of this series were separable from one another, they might form the pattern indicated. Mutants occurring in the overlap region would show both the (**o**) and (**v**) characters. Such an interpretation, analogous to one proposed by PONTECORVO (1955) for Aspergillus, would explain the allelic interaction in terms of the distance of the members from the site of maximal activity. This model would thus successfully account for the complementation (i.e., the normality of the *trans* combination or compound) of *'o/'v*, *'o/'lv*, and *'ol/'v*, and also for the extreme mutant appearance of *'olv/'ov*.

However, the localization tests of the pairs of mutants in the dumpy series did not confirm this point of view. As Figures 2 and 3 indicate, the relation between the mutants is not based on a phenotypic or obvious physiological gradient of ordered subloci, but appears, rather, to be scrambled. The region expressing the (**o**) phenotype is interrupted by *'lv*, and the region expressing the (**v**) phenotype is interrupted by *'o*, which also interrupts the region expressing the (**l**) phenotype. This interruption in continuity in the (**o**), (**l**), and (**v**) phenotypes along the map sequence demonstrates that pseudoallelism does not necessarily require a linear continuity of the functional elements expressed in a *trans* compound. It offers *prima facie* evidence for pseudoallelism of the lethal effect in this dumpy region.

The sequence *'o 'lv 'ov 'v* was established directly by obtaining six or more recombinants within *each* of the three intervals between the adjacent pairs, using the *ed* and *cl* markers to determine the left or right positions of the given mutants. The remaining members presented a difficulty. The pairs *'l/'ol* and *'ol/'olv* form lethal combinations and *'o/'olv* is too weak to breed effectively. An indirect method, employing recombination rates with the *'ov* sublocus as a standard of reference was therefore used. Since the difference in recombination rates between the *'ov*^{1} and either the *'l*^{m}, *'ol*^{s}, *'olv*^{1}, or *'o*^{2} subloci are believed large

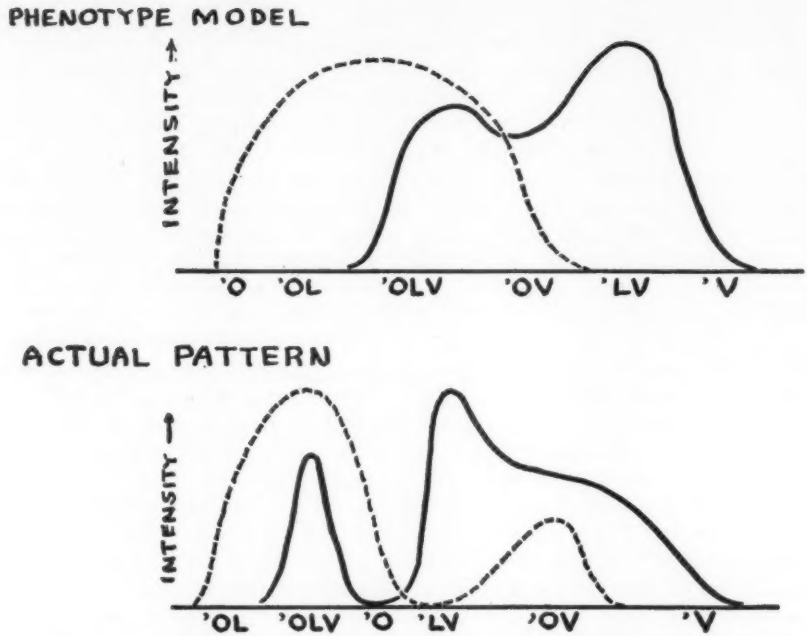

FIGURE 2.—The upper figure illustrates the order of subloci predicted on a direct relation between the intensity of mutant expression and the proximity, in terms of map distance between subloci for two overlapping gradients. The lower figure shows the type of quantitative and qualitative pattern which would occur if the actual order obtained showed a correspondence to such a phenotypically-determined physiological pattern.

enough to warrant their separate localization with respect to one another, and since two or more recombinants were obtained for each pair, these mutants are tentatively placed in the sequence *'l 'ol 'olv 'o* (see Table 3 for members involved and for recombination rates). Confirmatory crosses, based on the order established, were also made to test the linearity of these mutants in their subloci (Figure 3).

A test was also made with *'ov*^{n} and *'ov*^{51f} to see if these were alleles of *'ov*^{1}. Two series, *'ov*^{n}/*'ov*^{1} and *'ov*^{51f}/*'ov*^{1} were employed. No recombinants were obtained in approximately 66,000 and 51,000 flies counted, respectively. With 3.0 as the Poisson confidence limit for a null event at a probability of 0.05, the maximum size of the map regions for these two cases is 0.0091 and 0.0118, respectively, after correcting for the fact that only wild type recombinants can be detected. This is approximately the size of the minimum linkage value obtained in the region (0.011 map units), but one fifth the size of the distance between *'lv* and *'v* which are the neighboring subloci of *'ov*.

The possibility of another locus near *'ov* does exist. As seen in Table 3, dp^h lies between *'lv* and *'v*, hence it may either be an allele of $'ov^1$ or occupy a site near it. Although it shows an extreme (**ov**) phenotype, it is more nearly like *'ov* in its reactions than it is like *'olv*. It is nearly lethal as a homozygote, but is viable with *'lv* and other lethal members of the region. It is also more extreme in its (**o**) and (**v**) interactions with most of the other members than are any of the other *'ov* mutants (EDMONDSON 1954). In the compound, $dp^h/'ov^1$, it looks more like *'olv*/*'ov* than like a typical *'ov*. (See Figure 1 and Table 1 for phenotypic descriptions).

The more than one hundred recombinants within the region were found by breeding tests to be true to type and to conform consistently to the direction of the *ed* and *cl* markers. However, three exceptions, listed as reverse mutations, might alternatively be considered double crossovers or "gene conversions" occurring within this 5.5 map unit region between the markers. These three cases have the following origin:

(a) an *ed* + *cl* ♂ from $'lv^1/ed\ 'ov^1\ cl$ ♀ × *ed* $'ov^1\ cl$ ♂. Not a complete reversion, partially oblique and vortex, showing a near normal phenotype at 26°C as a homozygote, and a phenotype characteristic of *'ov*/*'ov* rather than of *'olv*/*'ov* (see Figure 1) when in *trans* combination with $'olv^1$.

(b) an *ed* + *cl* ♀ from $'ov^n/ed\ 'ov^1\ cl$ ♀ × *ed* $'ov^1\ cl$ ♂. Complete reversion; (+) with $'olv^1$.

(c) a wild type female from $'ol^m/ed\ 'ov^1\ cl$ ♀ × *ed* $'ov^1\ cl$ ♂. Complete reversion to nonlethal.

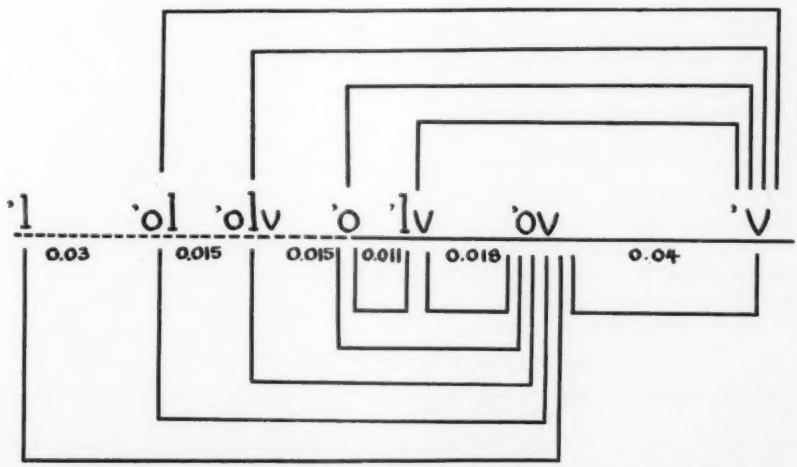

FIGURE 3.—Map distance relations of the seven mutant genotypes of the dumpy series. Dotted line indicates provisional relation is based on differences in recombination with *'ov*. Solid line indicates unequivocal direct recombination between adjacent subloci.

All of the above cases could be cases of suppressors consisting of mutants arising near or in the dumpy region itself and acting in a direction opposite to the mutant form (hypermorphic). Such an interpretation could only be ruled out by pseudoallelic analysis of the reverse mutations in an attempt to extract the original components. Furthermore, homozygous stocks of mutants may differ, not only from one another, but from their heterozygous compounds, in their mutation rates. Errors or differences of one order of magnitude may be obtained between forward mutations in such stocks (MULLER and ALTENBURG 1919) and there is no reason to suppose that the same does not hold true for reverse mutations. A second source of error in these studies would come from differences in reversions from the alleles themselves, even if the stocks were coisogenic for the rest of the genome. This has been illustrated for isoalleles of white and yellow in Drosophila (TIMOFEEFF-RESSOVSKY 1931 and LEFEVRE 1954) where considerable differences occur in their induced mutation rates.

For these reasons it seems that the precautions used by MULLER, *et al.* (1950) and by SCHALET (1957) in the determination of spontaneous mutation rates for selected loci on the X chromosome should be used in "conversion" studies. SCHALET used the spontaneous lethal frequency in the different stocks as an index of the mutability of the stock and as a control to determine the reliability of the rates for any such series. In addition, a critical analysis of the problem of "conversion" or reversion of mutations in Drosophila should use inversion heterozygotes or heterozygous males (where crossing over is absent) as a further control to rule out recombination as a mechanism in the exceptional reversions in such pseudoallelic regions.

The preliminary tests for allelism which indicate that *'ov*^{n}, *'ov*^{s11}, and *'ov*^{1} are alleles, seem to support the findings of GREEN and GREEN (1956) that discontinuities in map distance exist between subloci of a genetic region of related function. These discontinuities may represent either actual subgenes within which recombination does not occur or they may represent relatively small sites within a continuous gene in which clusters of specific mutations result as a consequence of their more frequent alteration at certain sites. These two interpretations are still open to further investigation for this region. The number of flies counted in an attempt to separate members of the *'ov* sublocus must, however, be considerably increased to distinguish between these theories since their separability by recombination would eventually be expected in a gene model that behaved like that of BENZER's for the *r*II region in phage.

Radiation analysis of the dumpy series

There was good reason to believe that the mutation rate in the dumpy region would be high enough, even at a low dose, to justify a detailed experimental analysis. This was partly because of FOGEL's original statement that the frequency of X-ray induced mutations in this region was high and partly because of the many spontaneous occurrences of these mutants in laboratories throughout the world. Whether or not the data obtained from such an investigation

would have any direct bearing on the structural interpretation of the dumpy region, it was believed that mutation rates and a mutational spectrum in this region would be of interest since this problem has not yet been sufficiently attacked in Drosophila.

For this experiment, Oregon-R wild type males, 2–4 days old, were permitted to inseminate, for 24 hours, virgin females of the composition *ed 'ov¹ cl*, of the same age as the males. The inseminated females were treated with X-rays at an intensity of approximately 250r per minute, giving a total cumulative dose of 1000r for a low dose and 4000r for a high dose. A separate control line was set up at the same time.

For equivalent crowding conditions, 60 females were used per bottle in the high dose series, 30 females per bottle in the low dose series, and 15 females per bottle in the control. These gave an over-all mean yield per bottle of 95 F_1 in the high, 327 F_1 in the low, and 311 F_1 in the control. Four broods were made, with intervals of 2, 2, 3, and 4 days respectively. A total of 11 days egg laying was permitted then, from the time of treatment to the removal of the parental females. All cultures were kept at 26°C to maintain optimum expression of the mutant phenotype. The summary results of this experiment are given in Table 4 and presented in graph form in Figure 4.

No striking difference was noted with respect to the broods, although the numbers were too small on a brood basis to justify any general conclusions. From the summary results, however, several conclusions can be made. First, of those mutants which were transmissible, irrespective of manner of origination, only two out of 24 at the high dose and only one out of 19 at the low dose represented mutations having single effects. Second, the extreme (**olv**) type mutant in both the high and low dose series occurred more frequently than any of the other mutant types. Third, at both high and low doses many mutants (about one third to one half the high and about one fifth of the low) were transmitted that had a mosaic origin. Fourth, the rate of production of these mutations by X-rays, when certain corrections are made, is linear with dose.

An analysis of the raw data presents many difficulties. At the high dose there were a number of (**olv**) type mutants which were too weak to breed and form stocks. There was only one such (**olv**) type at the low dose. It is assumed that these were weakened because they were deficiencies for the entire region, or that they were riddled with modifiers which intensified the mutant dumpy region in the compound and hence made it act as a lethal or semilethal. There is no reason to believe that these would not have been transmitted had they been able to breed.

In determining a mutation rate, or in comparing frequency dose curves, an error of about 25 percent would have to be introduced to take these inviable (**olv**) type high dose mutants into account. Another difficulty is the frequency of nontransmissible mutants, which constitute about 20 percent of the total induced mutations or dumpy phenotypes in the F_1. These cannot be counted in the determination of mutation rates because there is no means by which somatic muta-

TABLE 4

Induced mutations in the dumpy region

Phenotype	Full-body mutant *trans.*	Full-body mutant *nontrans.*	Mosaic-body *trans.*	Mosaic-body *nontrans.*	Inviable or sterile	Total	Rate/sperm/r
			High dose 4000r 14,650 F_1				
olv	8	0	3	1	8	20	34×10^{-8}
lv	5	0	2	0	0	7	12×10^{-8}
ol	0	0	1	0	2	3	5.1×10^{-8}
ov	2	0	1	1	0	4	6.8×10^{-8}
v	0	(1)	0	(2)	(1)	4	6.8×10^{-8}
o	1	0	1	2	0	4	6.8×10^{-8}
Totals	16	1	8	6	11	42	71.6×10^{-8}
			Low dose 1000r 49,704 F_1				
olv	9	0	3	3	1	16	32.2×10^{-8}
lv	3	0	0	0	0	3	6×10^{-8}
ol	2	1	0	0	1	4	8×10^{-8}
ov	1	0	0	3	0	4	8×10^{-8}
v	0	0	0	(7)	(1)	8	16.1×10^{-8}
o	1	0	0	2	0	3	6×10^{-8}
Totals	16	1	3	15	3	38	76.5×10^{-8}
			Control 31,154 F_1				
olv	0	0	0	3	0	3	
ol, lv / **ov, o**	0	0	0	0	0	0	
v	0	0	0	(2)	0	2	
cm	0	1	0	0	0	1	
Totals	0	1	0	5	0	6	

(Numbers in parentheses indicate that phenotype suggested *'v/'ov* rather than *'lv/'ov* genotype, the former being less extreme in expression.)

tions can be distinguished from various pleiotropic effects of the *'ov¹* chromosome in the heterozygote. Thus the incidence of (**v**) type mosaics which are nontransmissible is most likely to be interpreted as a penetrance of the heterozygous *'ov¹*. Curiously, too, the frequency of transmissibility among mosaic-origin mutants is much higher at the high dose than it is at the low dose.

The failure to obtain any transmitted spontaneous mutations in the control was probably due to a combination of circumstances. The count was relatively low for a spontaneous rate to be properly estimated and the six observed phenotypic exceptions (mostly of slight effect) were probably expressions of mutant penetrance in the heterozygote.

Crossover tests with the *ed* and *cl* markers were carried out on several of the surviving transmitted mutants and the results are summarized in Table 5. Out of 18 tested mutants, five were associated with rearrangements preventing crossing over with these markers. From the low dose, of 14 tested there were no rearrangements which prevented the *ed* and *cl* from crossing over. Thus approxi-

mately 25 percent of the high dose mutants which bred true and survived involved alterations large enough to be detected by this technique.

The data from the X-ray analysis thus supports the point mutation nature of the various alleles earlier considered, especially if we take into account the *'lv* mutants which are situated between *'o* and *'ov*. These occur with a relatively high frequency at both the high and low doses and could not likely have been due to minute rearrangements greater than 0.03 map units in length. The data only partially support the hypothesis that a high mutation rate in the dumpy locus is a consequence of a multiplicity of loci because two of the phenotypes, (**olv**)and (**lv**), constitute three quarters of the transmissible mutations. More-

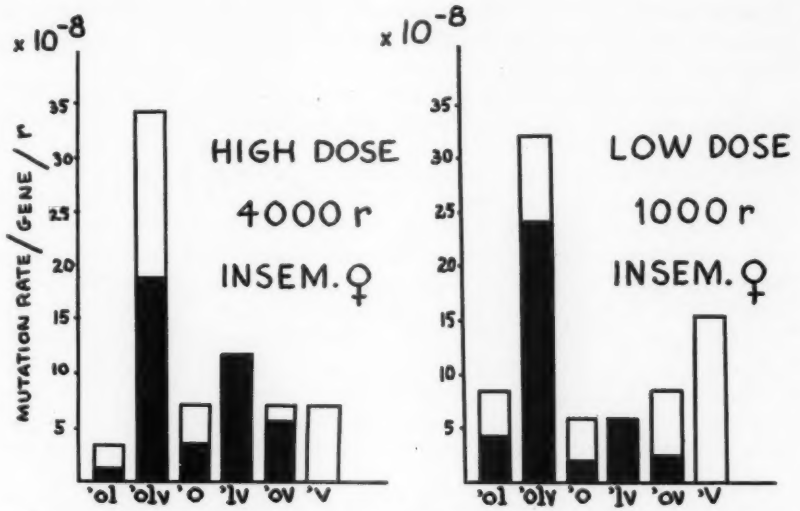

FIGURE 4.—X-rayed induced mutation rates from Oregon R wild type sperm. Black columns indicate mutants which bred true. White columns indicate sterile, pseudodominant, and fractional mutants which could not be followed up genetically.

TABLE 5

Crossing over tests of induced mutants

Mutant type	Number tested	Recombination in *ed-cl* region	Absence of recombination	Effect of added heterochromatin
olv	8	7	1	none
ol	1	1	0	none
lv	5	4	1	none
ov	2	0	2	wild phenotype
o	2	1	1	none

over, the rate even of the others (averaging 8×10^{-8}/r) is several times higher than that hitherto found for specific loci induced in Drosophila spermatoza.

Position effect and other interactions involving the dumpy region

From an experiment involving X-ray and neutron mutagenesis, SCHALET (1955, unpublished) found two exceptional wing mutants in the *trans* condition with *'lv*^{i} *Cy*. These were associated with rearrangements, one of them being a Y-2 translocation. These mutants, *dp*^{w} and *dp*^{w2}, studied by CARLSON (1955), showed the following features: in *trans* with *'ov*^{1} they showed a variegated expression of the (**o**) and (**v**) characters, with a very pronounced asymmetry for both of these effects; the homozygotes were inviable when attempts were made to make the second chromosome homozygous; and, in contrast to other known members of the series which carry a lethal effect, these rearrangements were viable with *'lv* and *'olv*.

This suggested the possibility that these were heterochromatic position effects associated with the rearrangements. A test was made using *dp*^{w3} males and X·Y *In En y; S 'ov*^{1} *Sp/'lv*^{i} *Cy* females. The offspring of this mating, all of which carried the extra Y chromosome, were completely normal for (**o**) and (**v**) characters. It was then possible, in the next generation, from an F_1 cross of *dp*^{w3}*/'lv*^{i} *Cy* sibs to obtain flies carrying the extra Y which were homozygous for *dp*^{w3}. Thus the lethal effect (which was not necessarily associated with that of the dumpy series) was also suppressed in this case by the use of added heterochromatin. Most of the homozygotes appeared wild type, but an occasional slight (**v**) effect was expressed in a few. It was also possible to test other rearrangements showing an asymmetric expression or variegation with added heterochromatin and these turned out to be suppressed also. On the other hand, those other rearrangements from the radiation analysis that did not show asymmetrical expression of the (**o**) and (**v**) phenotypes were not suppressed by this method. (See Table 5 for results.)

Another by-product of SCHALET's experiment concerns a transposition involving a segment of the second chromosome about three to four map units in length (from the *ed* locus to the scute-19 insertion, *ca.* 15 on the second chromosome map). This was inserted, probably into the third chromosome, of an independently arising X-3 translocation. This complex rearrangement is tentatively designated as: $\dfrac{y\ ac\ \text{TX3 Dp}\ (dp^{+});\ \text{Df}(dp^{-})}{\text{III}^{+} \qquad\qquad 'lv^{i}Cy}$.

The following facts have been determined from a preliminary investigation: (a) occasional homozygous *y ac* TX3 Dp (dp^{+}); Df(dp^{-}) females emerge and appear normal but are relatively infertile and cannot be used for matings; (b) the Dp(dp^{+}) covers the Minute bristle deficiency Df(2)*MB* for both its Minute bristle effect and the *ed* region, indicating the left break extends as far as *ed;* (c) the right break is in the scute-19 region since the TX3, when both of the Df(dp^{-}) second chromosomes are replaced, is able to cover the *ac* in the X chromosome. The phenotype of the exception itself was **y**, indicating that the

y^+ locus was lost or mutated in the process. If it was lost, it may have been at the edge of the break, in which case the scute-19 duplication was inserted in the *sc-ac-y* direction in the original second chromosome; (d) the hyperploid, carrying a single dose of the Dp(dp^+) is viable in both sexes but very infertile; (e) the hyperploid can cover a homozygous *'olv*^{1} or any of the pseudoallelic lethal combinations in *trans* (e.g., *'ol*^{s}*/'lv*^{1} or *'lv*^{1}*/'olv*^{1}) or even permit the homozygous Df(2)*MB* to live, suggesting that the Dp(dp^+) is at least as large, if not larger than this rearrangement.

It is possible to conclude from the above, and from other tests, that the members of the dumpy nest are hypomorphic and do not involve the production of abnormal substances which can successfully interfere, even in homozygous condition, with the normal functioning of a single dose of the dp^+ region, even when the latter is removed to a different chromosome environment.

Demonstration of weaker dumpy effects by the mutant members of the dumpy series

By the use of one of the Moiré mutants (*Mé*—II, 20, in both cases associated with a structural change) which, when present with certain dumpy alleles in the *trans* compound with dp^+, result in an (**o**) effect, it has been possible to establish the phenotypic relations shown in Table 6.

All of these mutants, with the exception of *'v*, show an (**o**) effect when Moiré is present, including the mutants *'l* and *'lv*, which do not show an (**o**) effect with the viable mutants having such an (**o**) element (e.g., *'o* and *'ov*)

TABLE 6

Effect of Moiré on the phenotype of dumpy alleles

	Genotype	Phenotype	Intensity	Comments
	'olv/+;Mé/+	**o**	+++	
	'ol/+;Mé/+	**o**	+++	
	'ov/+;Mé/+	**o**	++	
	'o/+;Mé/+	**o**	++	
	'lv/+;Mé/+	**o**	+	
	'l/+;Mé/+	**o**	+	
	'v/+;Mé/+	normal	none	
1	*'ol/'v;Mé/+*	**o**	+++	Indistinguishable
	'ol'v/+;Mé/+	**o**	+++	
2	*'o/'lv;Mé/+*	**o**(blisters)	+++++	Semilethal
	'o'lv/+;Mé/+	**o**(no blisters)	++	Viability normal
3	*'o/'v;Mé/+*	**o**	++	Indistinguishable
	'o'v/+;Mé/+	**o**	++	

when these are in the *trans* compound without Moiré. This suggests that the *'l* and *'lv* are able to express a weak (**o**) effect, but not sufficiently strong enough to show it without the Moiré intensifier, which somehow interferes with the normal effectiveness of the (**o**+) reaction.

A method was designed to test the effect of Moiré on the *cis-trans* phenomenon. As shown in Table 6, three series of compounds were used in which the *cis* and *trans* combinations of the same pairs of mutants were used with Moiré. Only in situation 2 did a difference between the two become very marked. The interesting aspect of this difference was not a greater degree of obliquity, but the blistering of the wings which became accentuated. The other series showed no *cis-trans* differences in the intensity of their (**o**) effect. These findings then, support the inference that *'v*^{2} has little effect on the (**o**) character.

An independent way of determining the weak effects within the region came out of analysis of the double recombinant mutants. The double recombinant *'o*^{2} *'v*^{2}, when homozygous, shows a much more extreme vortex effect than does homozygous *'v*^{2} alone. It is so much more noticeable that the usual higher temperature requirements for good expression of the (**v**) effect can be ignored and these double mutants will show the (**v**) effect at room temperatures in almost all cases. The effect on the wing is more variable, and shows the same widespread variation in size that homozygous *'o*^{2} itself does, but the more extreme members of *'o*^{2} *'v*^{2} are more extreme in their mutant appearance than are the most extreme members of *'o*^{2}. This can be demonstrated also by comparing the (**o**) character of *'o*^{2}/*'ov*^{1} in *trans*, which is less extreme than that of *'o*^{2} *'v*^{2}/*'ov*^{1}. A similar finding occurs in *'o*^{2} *'lv*^{1}/*'ov*^{1} which shows both a more extreme thorax and a more extreme wing effect than either of the two subloci of that double mutant with *'ov*^{1} in the *trans*. The same (**v**) intensification is seen in the *'ol*^{8} *'v*^{2}/*'ov*^{1} compound. These are suggestive that the presence of two mutant members in the region will result in an intensification of the (**o**) and (**v**) elements as a consequence of their additive or synergistic effects. That this principle extends to the lethal effect is indicated by the finding that *'o*^{2} *'lv*^{1}/*'o*^{2} *'v*^{2}, *'olv*/*'o*^{2} *'v*^{2}, and *'lv*^{1}/*'o*^{2} *'v*^{2} are lethal or nearly so, and *'ol*^{8} *'v*^{2}/*'o*^{2} *'v*^{2}, *'l*^{m}/*'o*^{2} *'v*^{2}, and *'ol*^{8}/*'o*^{2} *'v*^{2} are sublethal (having 0.5, 0.5, and 5.0 per cent viability, respectively). It seems plausible then that very weak pleiotropic effects can be brought out by the cumulative or synergistic activity of these double mutants in the *cis* configurations.

Another demonstration of the weak expression of the lethal effect which can be brought out in the *trans* state is seen in crosses made between stocks of the various complementary subloci having these balanced over the same Curly inversion. Thus in (a) *'o*^{2}/*Cy* × *'lv*^{1}/*Cy* gives very few (+) flies; however, in case (b) *'o*^{2}/*Cy* × *'v*^{2}/*Cy* gives a normal expectation of (+) flies, as does (c) *'ol*^{8}/*Cy* × *'v*^{2}/*Cy*. This suggests that *'o*^{2} has more of a lethal tendency associated with it than does *'v*^{2}.

Developmental aspects of the dumpy series

Pleiotropic effects may result from a single genetic product which acts in one or more reactions, simultaneously or at separate times in development. Alternatively, these pleiotropic effects may result from several products arising from a single gene at different times in development or by a group of closely linked subgenes, each having a specific substance affecting a specific one of the characters manifested pleiotropically.

In the dumpy locus it is possible to rule out a "one character—one subgene" interpretation (which would be analogous to that proposed in the FISHER-RACE theory for the C, D, and E antigens of the Rh series) since certain two-effect (*'ov*, *'ol*, *'lv*) or three-effect (*'olv*) mutations can be shown to occupy separate subloci without evidence of finer separability of their pleiotropic effects by recombination. (See Table 2.)

It is also possible to demonstrate that at least three stages of development are affected. Thus BLANC and CHILD (1937) found the (**o**) character expressed during the 13–14th hours of pupal development and the (**v**) character expressed 6–10 hours after pupation begins. In addition, the lethal effect does not express itself in a late larval or pupal stage and must therefore occur earlier (probably embryonic). This might be interpreted to mean that the same single product acts differently at three different times in development or, alternatively, that the dumpy gene acts in a different way for each of these developmental stages (as, for example, by having a structure whose reactive sites are capable of interaction only with corresponding specific substances which are available during these various developmental stages).

The weak effects expressed by these various subloci suggest that the entire gene is involved with alterations at certain sites eliciting a greater mutant response than others. However, the qualitative and quantitative differences for these characters in their diverse genotypic combinations also suggest that the mutant activities cannot be easily interpreted by simple models of gene action.

Genetic significance of the dumpy region

The dumpy region may originally have been composed of duplicate subgenes, presumably arising in the past by unequal crossing over or interchange between sister or homologous strands or, less often, by minute insertion, so that these duplicated segments of a genetic region were able to form an adjacent series (salivary "doublets"). (BRIDGES 1935; MULLER 1935a, b; LEWIS 1945, 1951). These would then have been able to mutate in independent ways and acquire new functions in time. Alternatively, its present parts may not have arisen as duplicates. Whatever its history has been, it now acts, in some respects at least, as a single but complex physiological unit, with certain sites of its chemical structure more likely to cause specific changes in phenotype, depending on the exact nature of the altered genetic region. This latter concept has been demon-

strated most effectively in bacteriophage, especially in the *r*II region of the *T* series viruses which attack *E. coli*. (BENZER 1955, 1957). Such a unit of physiological function, called a *cistron* by BENZER, seems in the phage to be continuous down to the nucleotide level, genetically and chemically. The distribution spatially of the individual mutants which occur within an *r*II cistron is a widely scattered one, and the various lesions resulting in a mutant effect are called *mutons*. That this phage cistron is too simple, as it stands, to account for the dumpy series is discussed later.

Although increases in genetic material are presumed to arise from duplications or repeats of small chromosome regions, their consequent hyperploidy should not result in a noticeable impairment of viability if these are to survive. In the historically important *Bar* case a position effect resulting in a neomorphic mutation accompanied the tandem duplication which brought it about (STURTEVANT 1925; BRIDGES 1936; MULLER 1936; MULLER, PROKOFEYEVA-BELGOVSKAYA, and KOSSIKOV 1936). The duplicational repeat and the position effect, however, are not necessarily related. The position effect, not only in Bar, but in several other loci in Drosophila, is of a more ubiquitous nature than one merely residing in the interactions of the repeat. Indeed, position effects, whether heterochromatic or euchromatic show a complicated relation in the type of change in phenotype expressed in a genetic region.

Minute alterations or even point mutations might occur (without exerting a phenotypic expression of their own) which nevertheless could exert a position effect on a neighboring locus (as suggested by OFFERMANN in 1935). Consequent recombination between such mutants exerting position effects and their true point mutation "alleles" at the locus susceptible to these effects, would result in an apparent recombination between alleles. This foreshadowing of pseudoallelism was based on a position effect interpretation alone, without necessitating a repeat formation for its occurrence. OFFERMANN further implied that recombination analysis might reveal *three* such positions for a single gene, depending on whether its two neighboring genes exerted position effects on it in opposite directions.

In the case of repeats that could give rise to hypomorphic mutants having a detectable phenotypic effect, there would also have to be an eventual loss of function in each of the adjacent members involved in order for a homozygous mutant effect to be produced. But such mutants would give just as much *cis* as *trans* effect unless positional relations influenced the result *in addition*. The achaete-scute series presents some of the problems encountered in a newly arisen duplication. A cytogenetic analysis of this general region was carried out by MULLER and his colleagues in a series of papers beginning in 1931 (see MULLER 1956 for a review of this work). The following high lights were brought out by the latter work: (1) radiation analysis by the "left-right" technique showed non random distributions of breakage regions. Sixteen breaks fell into five sites defining four genes, of which two, *ac* and *sc*, as mentioned in point (4) below, might be considered subgenes; (2) the entire region was excised by X-rays and inserted as a simultaneous deficiency-insertion into the second chromosome. It

was approximately one half of a double band in length on a salivary analysis by visible light, and even ultraviolet showed fewer bands than genes and subgenes; (3) the scute-19 insertion, as this transposition was called, survived as a hyperploid and hence could, in time, mutate independently. Usually such duplications are adjacent, as in Bar eyes, rather than transpositional, but the principle governing their future fate is the same; (4) an actual separation of ac^+ and sc^+ had already been achieved by SEREBROVSKY by X-ray techniques and both of these "subgenes" were able to function normally (and partially overlap each other's functions) when separated from one another by a considerable distance.

It is interesting to note that the best examples of presumably duplicated adjacent genes: achaete and scute, miniature and dusky, and the fertility genes of the Y chromosome, involve no *cis-trans* differences. On the other hand, those cases which best show a *cis-trans* difference have not yet been critically demonstrated by breakage to show their duplicated origin. Of course, if these cases are so interdependent that a physical separation would result in their total mutant expression, this technique would not be valid for the demonstration of the separateness of the members involved. As yet, therefore, no direct method is available for getting evidence of a duplicational origin in any such pseudoallelic cases.

What is really at the heart of the problem in these complex loci is the intragenic differentiation which can result in numerous allelic, pseudoallelic, and complementary expressions (see, for example, MULLER 1932). In the dumpy region this is seen not only in the diverse phenotypes expressed at seven subloci but also within one such sublocus region. Thus the mutants $'ov^1$, $'ov^R$, and $'ov^{51f}$ are similar in phenotype: but dp^h, which also seems to be in the same subgene, shows a quantitatively different phenotype. Because very small changes are involved in the lesions which result in mutations, it is not entirely impossible that qualitatively different mutations will also be found within the same subgene, giving the consequences of complementary alleles. Until there is critical experimental evidence to explain the discontinuities observed in these genetically related loci it would be premature to be committed to their mode of origin.

There are, however, ways of reconciling a duplicational interpretation with a loss of function of the subgenes when they are separated. The first functional model for a subgene interpretation of a pseudoallelic region, proposed by LEWIS in 1951 on the basis of his investigations of the Star-asteroid region, met this condition. The duplicated set was conceived to act as a single functional unit because of a sequential transfer of materials between the duplicated members. If one of these subgenes was mutant, the sequential transfer was blocked. Hence in the *trans* compound the two mutants, although occupying different subloci, prevented the normal functioning of the unmutated alleles in their respective chromosomes. (See Figure 5 for details of the "Lewis effect" as this interpretation has been called.)

A similar interpretation was offered by PONTECORVO in 1952 using a type of chemical reaction of such labile and transient quality that it required the cooper-

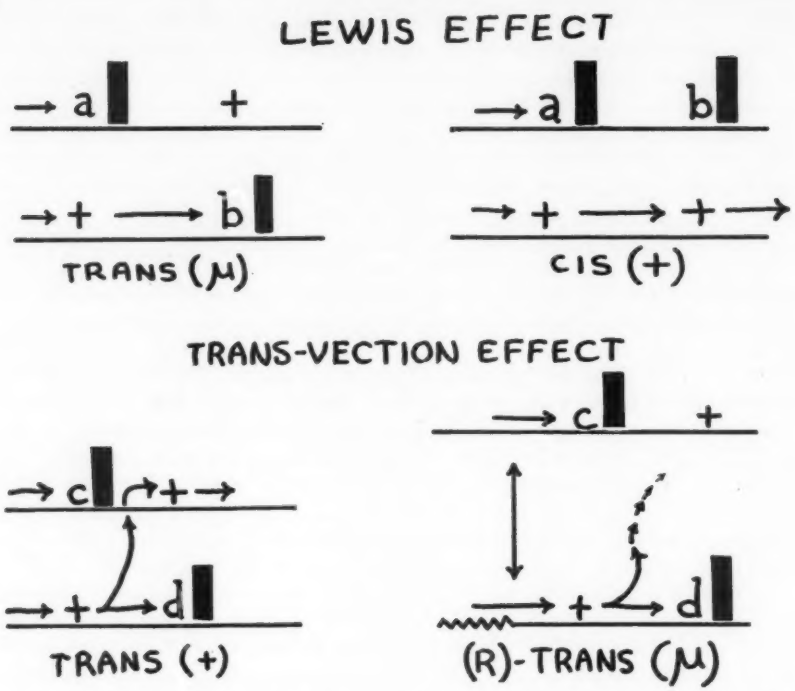

FIGURE 5.—In the "Lewis Effect" a *trans* pseudoallelic pair shows a mutant expression if the sequential transfer of precursors is blocked at any sublocus. A *cis* pair is complementary (wild type) because one chromosome permits the sequential transfer to go to completion. The "transvection effect" is defined for a *trans* pair which is complementary. The blocks in the sequential transfer are by-passed by a diffusion of the precursors from the normal pseudoalleles in one chromosome to the paired homologue. Rearrangements in either homologue may distort the pairing, reducing the concentration of the products diffusing across, and hence resulting in a mutant expression.

ation of very close genes to bring it to completion. These so-called "millimicromolar" reactions were considered characteristic of pseudoalleles.

Modifications of both of these theories have been made as new loci and new organisms have been studied. Thus LEWIS's later work (1955) with the bithorax series indicated a close pairing relation between the homologous chromosomes which was believed necessary for the normal functioning of this region. If certain mutant pairs which gave a well-defined difference in *trans* had rearrangements introduced into the arm of the third chromosome carrying the bithorax region, then the *trans* expression was usually more mutant than without such a rearrangement being present. Furthermore the breaks in these rearrangements could be present over a considerable distance from the *bx* locus. This implied a

distortion in the close pairing relations and hence a weakening of the transfer mechanism which permitted these regions to exchange products from one chromosome to another. (See Figure 5 for a diagrammatic representation of the "transvection" effect, as this phenomenon has been called.)

PONTECORVO found that most of the alleles which he subjected to crossover analysis in *Aspergillus nidulans* were able to show recombination. For this reason he believed the entire region investigated to be continuous, with the mutants representing lesions in the allelic "genes" carried by the homologous chromosomes. If these altered sites were close to one another, in terms of their map distance, then the allelic expression would be more extreme, but as the mutants showed alterations which were further apart with respect to one another, the mutant expression in the *trans* compound would be diminished. This implied a type of physiological pattern which could be based on phenotype and a diagrammatic representation of such a model in the dumpy series is shown in Figure 2 with the "actual" pattern based on map localization as well as mutant intensity shown below it. (See PONTECORVO 1955 for review of this model.)

In the dumpy series there seems to be compelling evidence for a considerable number of mutant sites. Do these represent subgenes (duplicated or not) or relatively small areas of high mutation rate within a single cistron in BENZER's sense? Certain facts are not easily dismissed in deciding whether to accept the latter model in the form in which it has been defined for phage. First, the failure to obtain recombinants between *'ov*^{1}, *'ov*^{n}, and *'ov*^{51} suggests that, unlike phage, a grouping of mutant sites or mutons occurs within this dumpy region. This discontinuity may not reflect the existence of a separate subgene nor, necessarily, that certain sites are much more mutable than others, but it may imply that recombination occurs within certain restricted areas within the gene. This would not be an altogether unlikely possibility since the recombinational process of phage is known to be different from that of higher organisms.

A second difficulty for the unqualified acceptance of a cistron interpretation is the occurrence of an *'o* and of an *'lv* which both appeared in the high dose series and which both failed to yield recombinations between the *ed* and *cl* markers or to be suppressed (normalized) by the addition of an extra Y chromosome. If one of the breaks really occurred in the dumpy region, an (**olv**) type of expression (i.e. complete inactivation) would have been expected. Because of the relatively high rate of induced mutations at the high dose, it is not inconceivable that these two rearrangements occurred independently of the mutations. If, however, they truly represent euchromatic rearrangements showing a stable position effect, in a manner analogous to scute and to Bar, and if their breaks could be demonstrated to be within the dumpy region, it would require a major reinterpretation of the *cis-trans* differences which occur since these might then be considered a set of independently functioning subgenes and their interactions would be very complicated to work out. Conceptually, also, the presence of allelism, complementation, and pseudoallelism in the same region makes the cistron a more

complex unit to define on a rigid *cis-trans* functional test. Finally, the possibility that the dumpy region covers the four bands near 25A in the salivary map of BRIDGES (1938) is suggested by comparison of *M2B*, *M2C*, and *'olv*^{r}. It is also suggested by the fact that, judging by the map distance, the dumpy nest is nearly one order of magnitude larger than the *y-ac-sc* region, when allowance is made for the great reduction in crossing over in the latter region (see DUBININ, SOKOLOV, and TINIAKOV 1937 and EDMONDSON 1952, for crossover relations in the *y-ac-sc* region). Thus, if the scute-19 insertion is about one half of a double band in length, the dumpy nest might conceivably occupy several bands. The size involved for such a region is far larger than those present in bacteriophage.

In recapitulation, although the *r*II region showed the same phenotype for any muton occurring within a cistron, the dumpy gene, on such a model, would require certain modifications. First, the mutational sites in terms of map distance would be narrow and restricted within the gene rather than forming a blending or merging pattern from one sublocus to another. Second, the various alleles would show phenotypes characteristic of the mutational site that was affected, and lesions in certain regions would not necessarily affect this gene's ability to function normally, or nearly so, for the other developmental roles in which it participates. These more restricted regions might be designated by the older term *subgenes* (without commitment to their duplicational origin).

That pairing or the transvection effect (see Figure 5) is not likely to play a major role in the complementation observed in this series is suggested by reference to the crosses in Figure 6. As illustrated, *'ol/'v* is (+); and if the normal "allele" of *'ol* permits its precursor substance to diffuse to its paired homologous region, it may be passed along sequentially to the *'v*$^{+}$ subgene and be further modified to give a normal product. Similarly, if in *'o/'lv* the *'o* chromosome received the normal precursor product from *'o*$^{+}$, then the sequential passage from *'o* to *'lv*$^{+}$ by-passes the block and permits a normal end product to be produced. However, if we consider the *trans* compound *'o/'ol* we should expect a normal expression since both chromosomes should be able to supply the necessary precursors to by-pass the blocks in each chromosome. This is not the case, since *'o/'ol* shows an extreme mutant (**o**) expression. Similarly, it can be shown that *in these same compounds*, the chromosomes carrying a (**v**) element do not achieve the complementation by simple transfer across the paired chromosomes without running into the paradox of a mutant (**v**) expression in the *'v/'lv* situation but a (+) expression for *'o/'lv* and *'ol/'v*. Furthermore, a preliminary attempt to obtain a trans-vection effect with *'o*^{s}*/'lv*^{i}, *InCyL* has not been successful.

The hypothesis employing the intact gene with the modifications previously suggested, would be sufficient to produce the wild type effect. Hence the *cis-trans* difference would reduce itself to a case in which two homologous genes are both altered at different sites in such a way that both are unable to function normally for at least one of the particular developmental functions characteristic

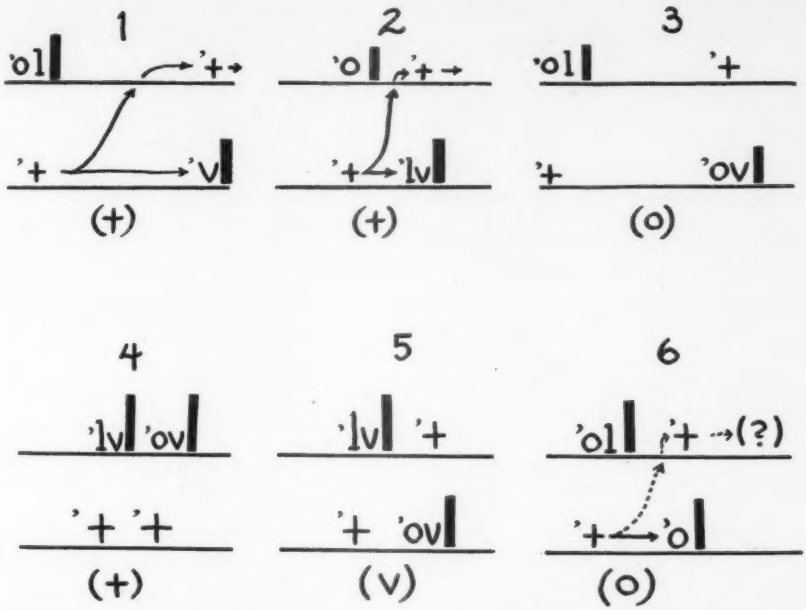

FIGURE 6.—In (1) the "trans-vection effect" is shown by-passing the block at *'ol*. In (2) the by-pass occurs at *'o*. In (6) the compound *'ol/'o* is mutant, not complementary, as would be expected if one or the other of these products diffused across. Items (4) and (5) demonstrate that typical "Lewis Effects" occur as well as the complementation seen in (1) and (2). Item (3) shows a *trans* pair with mutant expression; since these mutants are the left and right bounding subloci of the complementary pair in (2), and since the complementary pair (1) covers a larger map distance than the mutant pair (3), these relations illustrate the difficulty of a simple distance relation in accounting for the mutant or complementary expressions.

of the gene. This does not mean that they cannot function at all, since the complementary pairs in the dumpy series would indicate that a lesion in one region of a gene would not impair its activities for all of the phenotypic expression unless it occurred in a subgene controlling all these functions (e.g., like *'olv*). (See Figure 7).

SUMMARY

1. Four, and probably at least seven, subloci are present in the dumpy region.

2. The difference between the effects of the *cis* and *trans* combinations of these mutants does not seem to be directly related to the linear distance between subloci within the dumpy region nor to the exchange of products between paired homologous chromosomes.

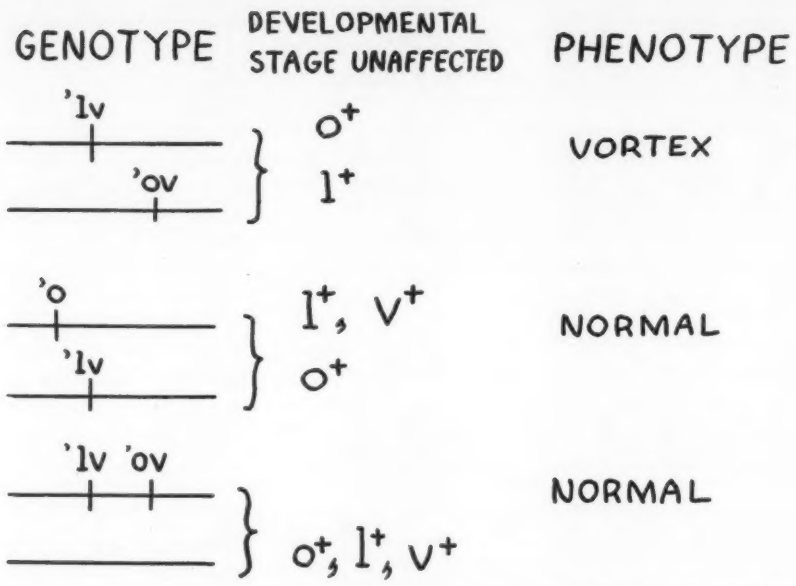

FIGURE 7.—The above gene model assumes that the dumpy gene can perform three acitivities associated with wing, thorax and viability expressions. The gene with an *'lv* alteration is able to provide a normal *o+* function; the gene with an *'ov* lesion has a normal *l+* reaction. Hence the (v) phenotype results since neither gene can act normally in thorax development. The *cis* compound *'lv 'ov/+* is normal because the intact gene operates normally on all three developmental levels. The complementary pair *'o/'lv* is normal since each gene is able to perform at least one of the three functions necessary for a normal phenotype.

3. Pseudoallelic subloci expressing a recessive lethal effect in the dumpy region are suggested.

4. Recombination has thus far not resolved the pleiotropic effects of individual subloci into finer subunits.

5. No suggestive evidence of "gene conversion" or aberrant recombination for this region has thus far been obtained.

6. Heterochromatic position effects associated with the dumpy region can be uniquely distinguished and demonstrated.

7. Mutation rates of the various mutant types differ considerably, with multiple effect phenotypes more common than single effect phenotypes.

8. Each mutant in the nest can be demonstrated to show at least a weak expression of the other remaining characteristics of this series by means of special modifiers and other genetic techniques.

9. The developmental processes resulting in the oblique, lethal, and vortex expressions of the dumpy series are separate in time.

10. A single gene model seems to provide a possible basis for this series,

although features are to be found in it (evidence for subgenes) that do not seem to be present in bacteriophage cistrons. Whether the dumpy subgenes arose by duplication from one another is still an open question.

ADDENDUM

Numerous contributions to the investigations of complex-loci have appeared since the first draft of this paper was written in February, 1958. The author regrets that space and time did not permit their analytical inclusion in this article. The interested reader is referred to a fuller and more up-to-date treatment of this problem in a review "Comparative Genetics of Complex Loci", Quarterly Review of Biology, March 1959, pp. 33–67.

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BISEXUAL MATING REACTION IN SACCHAROMYCES CHEVALIERI

TOSHIAKI TAKAHASHI[1] AND YONOSUKE IKEDA

Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan

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IN spite of the accepted belief that homothallic strains of Saccharomyces yeasts have no mating type, it was found by TAKAHASHI, SAITO, and IKEDA (1958) that the homothallic strain isolated by them (*Saccharomyces cerevisiae* S-341) does possess a mating type, but in a concealed form, and that its homothallism controlled by three pairs of alleles, e.g., strains possessing HM_1 HM_2 hm_3 and hm_1 HM_2 HM_3 are diploids, while those carrying HM_1 hm_2 hm_3, hm_1 HM_2 hm_3, and hm_1 hm_2 hm_3 are haploids (TAKAHASHI 1958).

This paper is concerned with studies on the mating reaction of *S. chevalieri*, and an attempt was also made to determine whether the diploidization gene *D* of this strain (WINGE and ROBERTS 1949) is equivalent to one of the *HM* genes. The material, *S. chevalieri* strain No. 9, was supplied from the Carlsberg Laboratory through the courtesy of DR. C. ROBERTS.

MATERIALS AND METHODS

Six strains were employed.

S-2141: A single spore culture of *S. chevalieri* strain No. 9; homothallic diploid carrying the gene *D*; adenine and arginine independent; galactose[2] and sucrose fermenter; maltose and melibiose nonfermenter.

714–41: A homothallic diploid carrying the genes HM_1 and HM_2; concealed mating type *a*; adenine independent and arginine dependent; galactose, sucrose, and melibiose fermenter; maltose nonfermenter.

753–44: A homothallic diploid carrying the genes HM_1 and HM_2; concealed mating type α; adenine and arginine dependent; galactose, sucrose, and melibiose fermenter; maltose nonfermenter.

733–51: A heterothallic haploid carrying the genes HM_1 and hm_2; mating type α; adenine and arginine dependent; galactose, maltose, and melibiose fermenter; sucrose nonfermenter.

733–53: A heterothallic haploid carrying the genes HM_1 and hm_2; mating type *a*; adenine and arginine dependent; sucrose, maltose, and melibiose fermenter; galactose nonfermenter.

H-965: A heterothallic diploid strain; heterozygous for mating type and maltose fermentation; homozygous for HM_1 and hm_2, adenine and arginine dependency, galactose and melibiose fermentation, and sucrose nonfermentation.

Crosses between diploid strains were performed by the spore-to-spore copula-

[1] Present address: Brewing Scientific Research Institute, Asahi Breweries Co. Ltd., Suita-shi.

[2] According to WINGE and ROBERTS (1948), *S. chevalieri* is a slow-fermenter.

tion technique (WINGE and LAUSTSEN 1938). The mating type of the haploid segregants was determined by the mass mating method involving the presence or absence of a mating reaction (LINDEGREN and LINDEGREN 1943); these types will be described hereafter as mass mating types in contrast to the concealed mating types, which were detectable only by the minimal plate mating method (TAKAHASHI, SAITO, and IKEDA 1958).

Nutritional requirements were determined by the presence or absence of positive growth on minimal medium supplemented with adenine (20 mg/1000 ml) and/or arginine (100 mg/1000 ml). Sugar fermentation was tested by the use of B. C. P. indicator medium (HAWTHORNE 1955).

Modified Lindegren's yeast broth (see SAITO and TAKAHASHI 1957), Burkholder's medium (see LINDEGREN 1949), and FOWELL's medium (FOWELL 1952) were used as complete, minimal, and sporulation media, respectively.

Genotypes are to be expressed in the following way: homothallism (diploidization) controlling gene in *S. chevalieri*, *D*; homothallism controlling complementary genes in strains 714–41 and 753–44, HM_1, HM_2; mating type, a/α; independence/dependence of adenine and arginine, *AD/ad* and *AR/ar*; ability/inability to ferment galactose, sucrose, maltose, and melibiose, *GA/ga*, *SU/su*, *MA/ma*, and *ME/me*.

RESULTS

Hybrids H-891 and H-899 were produced by the spore-to-spore copulation technique.

H-891:	714–41	HM_1	HM_2		*a*	*AD*	*ar*	*GA*	*SU*	*ma*	*ME*
	S-2141			*D*		*AD*	*AR*	*GA*	*SU*	*ma*	*me*
H-899:	753–44	HM_1	HM_2		α	*ad*	*ar*	*GA*	*SU*	*ma*	*ME*
	S-2141			*D*		*AD*	*AR*	*GA*	*SU*	*ma*	*me*

In the tetrads arising from these two hybrids, every character except sexuality showed a 2:2 segregation as seen in Table 1 and Table 2. With regard to the sexual behavior, the single spore cultures could be classified into three types, as follows:

Type I: The strains showed positive mass mating reaction and negative sporulation. They are heterothallic haploids carrying either HM_1 or HM_2.

Type II: The strains showed negative mass mating reaction, positive sporulation, and positive concealed mating reaction. They mated with either *a* or α only when the minimal plate mating method was employed. They are homothallic diploids carrying *aa* or $\alpha\alpha$ and HM_1HM_1, HM_2HM_2.

Type III: The strains showed negative mass mating reaction, positive sporulation, and positive concealed mating reaction. They mated with both *a* and α only when the minimal plate mating method was employed. They are homothallic diploids probably possessing *DD*, and they are bisexual.

Through tetrad analysis, the hybrids were found to segregate out as either two Type III and two Type II, or as two Type III, one Type II, and one Type I.

TABLE 1

Segregation data of H–891

Segregant	Segregation	Sexual behavior			
		Mass mating type	Concealed mating type	Sporulation	Type
891–11	*ar me*	–	*b**	+	III
12	*AR me*	–	*a*	+	II
13	*AR ME*	–	*b*	+	III
14	*ar ME*	*a*	– †	—	I
891–21	*ar ME*	–	*a*	+	II
22	*AR me*	–	*b*	+	III
23	*AR me*	–	*b*	+	III
24	*ar ME*	–	*a*	+	II
891–31	*AR me*	–	*b*	+	III
32	*ar me*	–	*b*	+	III
33	*ar ME*	–	*a*	+	II
34	*AR ME*	–	*a*	+	II
891–41	*AR ME*	–	*b*	+	III
42	*AR me*	–	*a*	+	II
43	*ar me*	*a*	–	—	I
44	(nongerm.)				
891–51	*AR ME*	–	*b*	+	III
52	*ar me*	–	*a*	+	II
53	*AR me*	–	*b*	+	III
54	(nongerm.)				

* Bisexual.
† Not examined.

Because the concealed mating types of strains 714–41 and 753–44 were unisexual, the bisexuality must have arisen from strain S-2141.

In order to test this hypothesis, the concealed mating potency of strain S-2141 was re-examined. This strain was positive in sporulation and reacted with neither *a* or *α* by the mass mating method, but it mated with both *a* and *α* by the minimal plate mating method. Moreover, the sister spore cultures which arose from the same ascus were also bisexual. Therefore, the original culture of *S. chevalieri* strain No. 9 is apparently homozygous with regard to bisexuality.

Assuming that bisexuality might be related to the presence of the *D* gene, a new hybrid (H-1162) was produced by the spore-to-spore copulation technique.

H-1162: $\dfrac{\text{S-2141} \quad D\ AD\ AR\ GA\ SU\ ma\ me}{\text{H-965} \quad HM_1\ hm_2\ ad\ ar\ GA\ su\ MA\ ME}$

Only two ascospores in each ascus of H-1162 germinated normally, and every character except mating type segregated out in general in a 1:1 ratio. Mating types of the single spore segregants were either unisexual by mass mating or bisexual by minimal plate mating as seen in Table 3. None of the segregants

belonged to Type II; this was expected due to the absence of the HM_2 gene in this material.

Hybrids H-981 and H-986 were produced by crossing cells of S-2141 with cells of 733–51 and 733–53 according to the minimal plate mating method. The cell sizes of the resulting hybrids are larger than any of the parental strains (Table 4 and Figure 1), and they seem to be triploids. Their segregation pattern will be reported elsewhere.

DISCUSSION

As stated before, the starting strain S-2141, a single spore culture of *S. chevalieri*, is homozygous with regard to bisexuality; it mates with cell of both *a* and *α* strains of heterothallic haploids as well as with spores of concealed *aa* and *αα* strains of homothallic diploids. Therefore, this strain of *S. chevalieri* is bisexual both in the ascospore state and the vegetative cell state.

All of the diploid segregants obtained from H-1162 were bisexual. Consequently, gene *D*, which was reported by WINGE and ROBERTS (1949) as responsible for the self-diploidization of single ascospore cultures in *S. chevalieri*

TABLE 2

Segregation data of H–899

Segregant	Segregation	Sexual behavior			
		Mass mating type	Concealed mating type	Sporulation	Type
899–11	*ad ar me*	–	*b*	+	III
12	*AD AR ME*	*a*	–	—	I
13	*ad ar me*	–	*α*	+	II
14	*AD AR ME*	–	*b*	+	III
899–21	*ad ar ME*	–	*α*	+	II
22	*ad AR ME*	–	*b*	+	III
23	*AD AR me*	–	*b*	+	III
24	*AD ar me*	–	*α*	+	II
899–51	*AD ar ME*	*a*	–	—	I
52	*AD AR ME*	–	*a*	+	II
53	*ad AR me*	–	*b*	+	III
54	*ad ar me*	–	*b*	+	III
899–61	*AD AR me*	–	*b*	+	III
62	*ad ar ME*	–	*b*	+	III
63	*AD AR me*	*α*	–	—	I
64	(nongerm.)				
899–71	*AD AR me*	*a*	–	—	I
72	*ad AR ME*	–	*b*	+	III
73	*AD ar me*	–	*α*	+	II
74	*ad ar ME*	–	*b*	+	III

TABLE 3

Segregation data of H–1162

Segregant	Segregation	Sexual behavior: Mass mating type	Concealed mating type	Sporulation	Type
1162–31	*ad AR su me*	–	*b*	+	III
32	*ad AR su ME*	*a*	–	—	I
1162–41	*AD AR SU ME*	*a*	–	—	I
42	*AD ar su me*	–	*b*	+	III
1162–51	*AD AR su ME*	*a*	–	—	I
52	*AD ar su me*	*a*	–	—	I
1162–61	*AD AR su me*	–	*b*	+	III
62	*AD AR SU ME*	*a*	–	—	I
1162–71	*AD AR SU me*	*a*	–	—	I
72	*AD ar SU ME*	*a*	–	—	I
1162–81	*ad ar SU me*	–	*b*	+	III
82	*ad ar SU ME*	*a*	–	—	I
1162–101	*ad ar SU ME*	–	*b*	+	III
102	*ad ar SU me*	–	*b*	+	III

TABLE 4

Check of marker traits as evidence for conjugation between S–2141 and haploid strains

Strain	Genotype or phenotype								Cell size (volume)
733–51	*a*	*d*	*ad*	*ar*	*GA*	*su*	*MA*	*ME*	4.35μ × 5.25μ (52.2μ³)
733–53	*a*	*d*	*ad*	*ar*	*ga*	*SU*	*MA*	*ME*	4.40μ × 5.25μ (52.8μ³)
S–2141		*D*	*AD*	*AR*	*GA*	*SU*	*ma*	*me*	3.88μ ×12.60μ (128.8μ³)
		D	*AD*	*AR*	*GA*	*SU*	*ma*	*me*	
H–981 $\left(\frac{733\text{–}51}{S\text{–}2141}\right)$			*ADE*	*ARG*	*GAL*	*SUC*	*MAL*	*MEL*	5.85μ × 10.45μ (161.4μ³)
H–986 $\left(\frac{733\text{–}53}{S\text{–}2141}\right)$			*ADE*	*ARG*	*GAL*	*SUC*	*MAL*	*MEL*	5.65μ × 9.40μ (156.1μ³)

might have something to do with the mating potency. One possibility is that this gene converts both mating types to a neutral type (n), which is able to mate with both a and α types. If one assumes that the following cross is made:

$$nD \times ad$$

then the meiotic products of the hybrid could be:

(1) $aD \rightarrow aaDD$
(2) ad
(3) nd
(4) $nD \rightarrow nnDD$.

As matter of fact, the diploids were all bisexual. Therefore if this hypothesis is correct, *aaDD* must have been converted into *nnDD*. To explain the rather frequent appearance of the α mating type in this material, transformation from

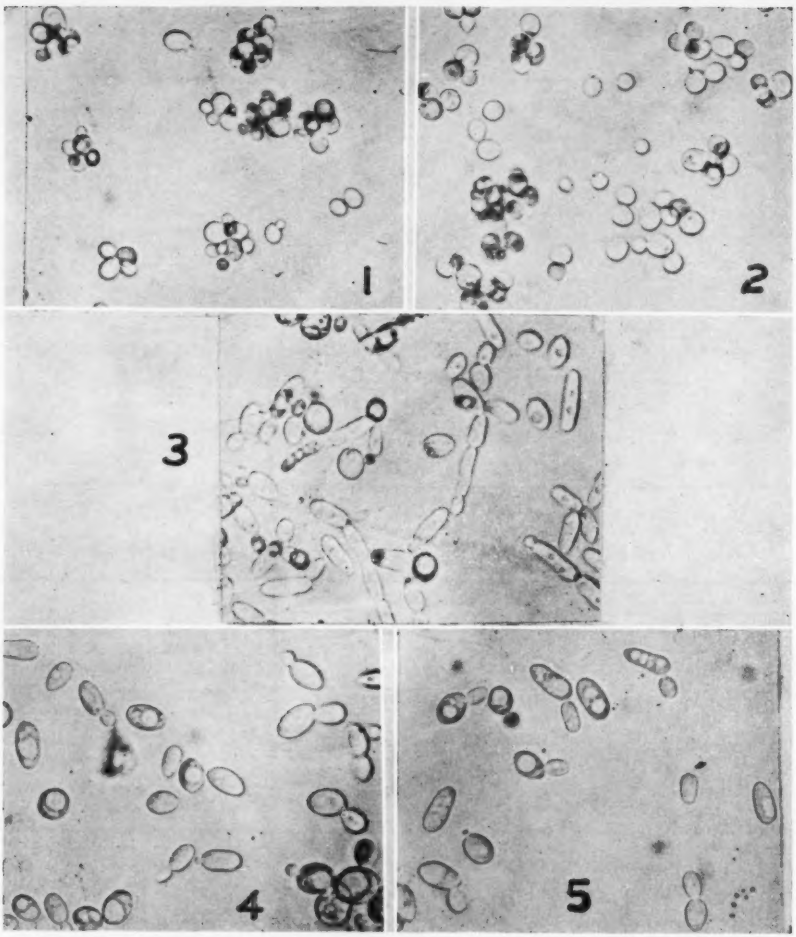

FIGURE 1.—Assumed triploids and their parents. (1) haploid: 733–51. (2) haploid: 733–53. (3) diploid: S–2141. (4) triploid: H–981 (733–51 × S–2141). (5) triploid: H–986 (733–53 × S–2141).

nd to *ad* must also be taken into account. However, since here the accompanying gene is *d*, rather than *D*, and since gene *n* is considered as neutral, the transformation might possibly take place.

In connection with this hypothesis, the authors wish to refer to WINGE and ROBERTS's statement (1958), according to which the ascospores arising from an *aaDD* culture were able to mate with every ascospore from an *aaDD* culture;

aD thus mated with both *aD* and *αD*, and this fact is consistent with the present view. However, the above hypothesis will be studied further.

None of the *HM* genes appear to be identical with the gene *D*. Strains with HM_1 hm_2 hm_3 and hm_1 HM_2 hm_3 are haploids and strains with HM_1HM_1 HM_2HM_2 hm_3hm_3 and hm_1hm_1 HM_2HM_2 HM_3HM_3 are diploids and either *a* or *α*. However, as mentioned previously (TAKAHASHI, SAITO, and IKEDA 1958) homothallic strains with *HM* genes were sometimes asexual by the minimal plate mating method, and, in addition, the sexual reaction of polyploid yeasts becomes weaker with increase in ploidy, so that it might be supposed that the diploidization genes such as *D* and *HM* could produce a common effect upon the expression of mating potency.

As seen in Table 1 and 2, a few heterothallic haploid cultures were produced from the hybrids between two homothallic Saccharomyces. This may be due to the appearance of segregants carrying either the HM_1 or the HM_2 gene, because the crosses were made between strains with HM_1 HM_2 and strains with hm_1 hm_2. Thus the production of heterothallic yeasts from the homothallic yeasts is indicated.

SUMMARY

In order to examine whether or not the concealed mating type found in a homothallic strain of *S. cerevisiae* is common among another species of homothallic yeasts, the mating potency of *S. chevalieri* was studied.

It was found that this yeast is bisexual, being able to mate with both *a* and *α* strains in the ascospore and the vegetative cell states. Among the segregants from the hybrids between a *D* gene strain and a Carbondale heterothallic strain, the diploids were invariably bisexual. It was concluded that the *D* gene was different from any of the *HM* genes, and its connection with bisexuality was discussed.

Heterothallic haploid cultures were produced from the hybrid between two homothallic Saccharomyces. These haploid cultures were presumed to carry either the HM_1 or the HM_2 gene.

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